

The role of *Tarebia granifera* as an invasive in freshwater ecosystems of southern Africa

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Summary

Over the past few decades, the increase in import and trade frequency of exotic aquatic species for aquarium hobby sales have led to the introduction and establishment of several invasive snail species on a global scale. Species become invasive when they are introduced and become established in environments outside of their native ranges where they can cause environmental harm. Invasive species are often responsible for a loss in native biodiversity that result in disrupted ecosystem function. Freshwater snails are considered one of the most endangered groups of species. The main threat to native snail populations is the spread of invasive snail species that have been reported to cause declines and extinctions in native snail populations globally. The establishment of invasive species is one of the major threats to freshwater biodiversity in South Africa. It is therefore of national priority that invasive species should be monitored and managed in order to protect the native biodiversity.

The freshwater snail *Tarebia granifera* (Lamarck, 1822), originally from Southeast Asia, is an invasive aquatic snail on three continents, including Africa as well as North and South America. The snail was first reported in South Africa in 1999 from the northern parts of the KwaZulu-Natal province and it has since rapidly spread into the Mpumalanga and Limpopo provinces, including several important conservation areas within the country. The snail is known to become the dominant species within invaded ecosystems and can reach densities of well over the thousands of individuals/m². The rapid spread and high densities reached by this invader have raised concerns regarding the possible negative impacts it may have on native snail species, primary benthic production and the trophic dynamics within invaded ecosystems. Previous research has investigated the effects of *T. granifera* in the coastal lakes and estuaries of KwaZulu-Natal. However, knowledge of the current distribution of this invader and its possible environmental impacts on the aquatic biodiversity and community structures of freshwater ecosystems are understudied. Furthermore, *T. granifera* is well known to act as the first intermediate host for diverse and prevalent species of disease-causing trematodes within and outside their native ranges and there is a need to investigate its role as a host or diluter for such parasites in South Africa.

The first aim of this study was to investigate the current distribution, densities, and population size structures of *T. granifera* in the Limpopo River system and Phongolo River in South Africa. Each of the 19 selected sites were sampled for water quality and aquatic benthic snails, following standard methodologies. All sampled snails were identified, measured and counted in order to calculate snail density/m² and determine snail population size structures. No significant correlations were found between any of the water quality variables and *T. granifera* density during

this study. It was further found that *T. granifera* had higher new-born recruitment during the spring and summer months when environmental conditions were more favourable. Molluscs were present at 14 of the 19 selected sites with a total of four species sampled, two invasives: *T. granifera* and *Physella acuta* (Draparnaud, 1805), and two natives: *Melanoides tuberculata* (Müller, 1774) and *Corbicula fluminalis* (Krauss, 1848). *Tarebia granifera* was usually the dominant species and the only species sampled from all 14 sites, making up 89% of all collected mollusc samples. Empty shells of three native snail species (Planorbidae sp. Rafinesque, 1815, *Bulinus tropicus* Krauss, 1848, and *Bulinus forskalii* Ehrenberg, 1831) were also found at sites where *T. granifera* dominated. In addition, historical records indicated that numerous native aquatic snail species from the sites sampled during the present study were also missing. *Tarebia granifera* seem to have displaced these native snail species from these invaded ecosystems.

As primary producers, diatoms play a key role in aquatic ecosystems as they are a food source for numerous heterotrophic species such as grazers and filter feeders. Several studies suggested that *T. granifera* can significantly reduce algal standing stocks, with unknown implications on aquatic consumers and higher trophic levels. Therefore, the second aim of this study was to determine the effects of *T. granifera* on benthic diatom community structures at selected *in situ* sites on the Phongolo, Olifants and Limpopo rivers. This aim was achieved by sampling diatoms from the same sites and sediment surfaces where the snails were sampled from. Benthic diatom samples were collected in triplicate from each site, following standard methodologies. The diatoms were identified to the lowest possible taxonomic level and divided into functional groups. A density scale was developed for *T. granifera* sampled from the selected sites: None, Low, Medium, High and Very high. With the use of a variety of univariate and multivariate statistical analyses and diversity indices, the possible effects of *T. granifera* density on benthic diatom community structures from the selected sites were assessed. Key findings were that *T. granifera* alters benthic diatom community structures, at high densities. Benthic diatom communities with high densities of *T. granifera* had an overall lower benthic diatom species diversity and disrupted functional groups with changes in diatom species abundance and dominance patterns. This was likely due to the high grazing pressure and bioturbation caused by the high densities of *T. granifera* at these sites. Sites with lower densities of *T. granifera* and the one site dominated by the native *M. tuberculata* had higher benthic diatom diversity, more diatom taxa, higher species richness and benthic diatoms that contributed to different diatom functional groups. Benthic diatom functional group analysis indicated that *T. granifera* may feed more on certain groups of benthic diatoms than others, leading to shifts in benthic diatoms dominance patterns, with higher abundances of diatom species (such as *Cocconeis*, *Gomphonema* and *Fragilaria*) with characteristics that enable them to resist *T. granifera* grazing pressure. Results also indicated that *T. granifera* size may also play a role in their diet preference, as sites with high densities of

small *T. granifera*, had higher abundances of large diatom groups than sites with high densities of larger *T. granifera*.

The effects of *T. granifera* on benthic diatoms were then further investigated with the use of an experimental microcosm study on the Phongolo River Floodplain. Nine microcosms were filled with water (540 litres) from the Phongolo River, as well as fifty kilograms of dry floodplain sediment. The microcosms were randomly divided into three treatment groups. The first treatment group were seeded with *T. granifera*, the second with the native *M. tuberculata* and the last group was free of snails. Snails were seeded to represent the average density from the sites on the Phongolo River. Water and benthic diatom samples were collected in triplicate from each of the microcosm treatments five weeks after snail seeding, following standard methodologies. A variety of univariate and multivariate statistical analyses and diversity indices were used to assess differences in community structures. Results from this study indicated that microcosms containing *T. granifera* had lower benthic diatom diversity, fewer individuals and diatom species representative of the earlier stages of the colonisation process, leading to altered benthic diatom community structures and functionality. Microcosm treatments without *T. granifera* had more evenly distributed and diverse benthic diatom species and functional groups, with diatom species representative of the later and final stages of the colonisation process. From both the *in situ* river and experimental microcosm studies, it can be concluded that *T. granifera* poses a threat to native biota by disrupting community structures of benthic primary producers within invaded aquatic ecosystems.

The final aims of this study were to investigate the role of the invasive thiarid (*T. granifera*) versus the native thiarid (*M. tuberculata*) as hosts for parasitic trematodes within the Limpopo River system and Phongolo River and to determine the genetic diversity of *T. granifera* from the selected sites. *Tarebia granifera* and *M. tuberculata* were collected from the selected sites and the infection status of the snails was tested with cercaria shedding experiments to induce the release of trematode cercaria. None of the snails shed cercaria during any of the shedding experiments and ten snails per species per site were randomly selected to determine prepatent trematode infections with the use of molecular analyses. Snails were dissected, whereafter, the hepatopancreas and a sample of each snail's foot tissue were removed. Snail hepatopancreas samples were used in order to determine trematode infections with the use of molecular (ITS2 and 28S) analyses. Snail foot tissue samples were used to characterise snails by molecular (*cox1*) and phylogenetic analyses. All dissected *T. granifera* were found to be females with embryos in their brood pouches and all *T. granifera* were found to be free from trematode infection within the study area. However, *M. tuberculata* was found to be infected with two species of trematodes. This study provides the first report of *M. tuberculata* as an intermediate host for *Haplorchis* sp.

and *Centrocestus* sp. in South Africa. This study further found all *T. granifera* from the selected sites on the Limpopo River system and Phongolo River to be genetically identical based on mitochondrial DNA (*cox1* gene). Furthermore, polygenetic analysis of *T. granifera* indicated that sequences obtained from *T. granifera* during the present study were genetically identical to sequences from China and Thailand. Thus, the snail was likely introduced into South Africa from one of these two countries.

From these results it can be concluded that *T. granifera* likely increase reproductive output during favourable environmental conditions that enables them to reach high densities and quickly become the dominant snail species. At high densities, *T. granifera* poses a threat to native biota by displacing native snail species and disrupting benthic diatom community structures, leading to reduced aquatic biodiversity and disrupted aquatic ecosystem function. Therefore, there is a need to control this invader to avoid further degradation of other freshwater habitats and aquatic biodiversity. This study also provides further evidence that the biodiversity of parasitic trematodes in thiarid snail hosts are understudied in South Africa and that there is a need for future research on these snail hosts and their parasites. Knowledge of the population dynamics and impacts of *T. granifera*, as well as their role as hosts for parasites, may aid in the development of management plans that may prevent the spread and destruction of these invaders on our native aquatic biota and their ecosystems.

Key words: *Tarebia granifera*, invasive snail, benthic diatoms, aquatic biodiversity, trematodes, invasion ecology, microcosm, freshwater ecology, genetics

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Chapter 1: General introduction

1.1 Background

The introduction of alien species is considered the third most important threat to freshwater biodiversity in southern Africa after habitat modification and pollution (Smith *et al.*, 2009; Weyl *et al.*, 2020). Knowledge of the taxa that are present, and their distribution in South Africa should be monitored as management and control of invasive species are of national priority (Weyl *et al.*, 2020; Makherana *et al.*, 2022a). Alien or non-native species refer to the introduction of species into new environments, outside their native range (Facon *et al.*, 2006; Richardson & Pyšek, 2006). Some alien species have the ability to adapt, survive and reproduce in new environments where they become established and often rapidly increase their population (Pimentel *et al.*, 2005; Richardson & Pyšek, 2006; Prentis *et al.*, 2008). Invasive species are defined as alien species that have been introduced, became established and spread while often causing harm to the environment and economy (Pimentel *et al.*, 2005; Richardson & Pyšek, 2006).

Despite their wide distribution and diversity, freshwater snails are one of the most endangered groups of species (Bogan, 2007). According to Almeida *et al.* (2018), the main threat to native freshwater molluscs is the introduction of exotic species. The aquarium industry, especially the aquarium hobby sales and the trade in aquatic plants, is responsible for freshwater snail introductions around the world (Walker, 1978; Madsen & Frandsen, 1989; Cowie, 1998; Pointier, 1999; Letelier *et al.*, 2007; Strayer, 2010; Preston *et al.*, 2022). In South Africa, 13 alien freshwater snail species have been introduced and seven of these species have become established, while up to 40% of the introduced freshwater snail species listed by Appleton (2003) have become invasive (Appleton & Miranda, 2015). Almost all freshwater snail species introduced before 1990 originated in the Americas, apart from an Australian species (*Physastra gibbosa* Gould, 1846) and one East African species (*Biomphalaria angulosa* Mandahl-Barth, 1957) that were unable to survive (Appleton, 2003; Appleton & Miranda, 2015). After the introduction of *Tarebia granifera* (Lamarck, 1822) from Southeast Asia in around 1996, two additional Asian species, *Radix rubiginosa* (Michelin, 1831) and *Gyraulus chinensis* (Dunker, 1848), were introduced in South Africa (Appleton & Miranda, 2015). According to Appleton and Miranda (2015), South African Revenue Service data indicated that imports from Asia doubled from 1998 to 2011. This increase in import frequency is responsible for a higher possibility of alien species introduction, and better control is required to prevent future introductions (Appleton & Miranda, 2015).

Tarebia granifera, also known as the quilted melania (Figure 1.1), is a prosobranch gastropod native to Southeast Asia (Abbott, 1952; Appleton *et al.*, 2009; Appleton & Miranda, 2015). It has become invasive in several subtropical parts of countries worldwide (Figure 1.2a-c), including the Caribbean islands, Texas, Mexico, Hawaii, Israel, Mozambique, Zimbabwe, Eswatini and South Africa (Chaniotis *et al.*, 1980a; Appleton, 2003; Pointier *et al.*, 2003; Appleton *et al.*, 2009; Karatayev *et al.*, 2009; López-López *et al.*, 2009; Miranda *et al.*, 2010; Appleton & Miranda, 2015). This species of snail was first reported in Africa by Appleton and Nadasan (2002) from the northern parts of the South African province of KwaZulu-Natal in 1999, where it was discovered in a concrete reservoir at Mandeni. *Tarebia granifera* was most likely introduced into the country as a stowaway of the aquarium industry (Picker & Griffiths, 2011). Since its discovery, it has now spread into several subtropical rivers and estuaries in southern Africa and was reported through KwaZulu-Natal northwards into the Mpumalanga and Limpopo provinces of South Africa (Figure 1.2d; Appleton *et al.*, 2009; Miranda *et al.*, 2011b; Malherbe, 2018). Appleton *et al.* (2009) collected *T. granifera* in 2006 from 13 rivers, five lakes, two dams, and several estuaries in northern KwaZulu-Natal. Well established populations have since also been found in the rivers of important conservation areas such as the Kruger National Park, iSimangaliso Wetland Park and Ndumo Game Reserve (Appleton, 2003; Wolmarans & de Kock, 2006; Dube *et al.*, 2017).



Figure 1.1: *Tarebia granifera* on filamentous algae in the Olifants River, South Africa. Photo credit: Prof N.J. Smit.

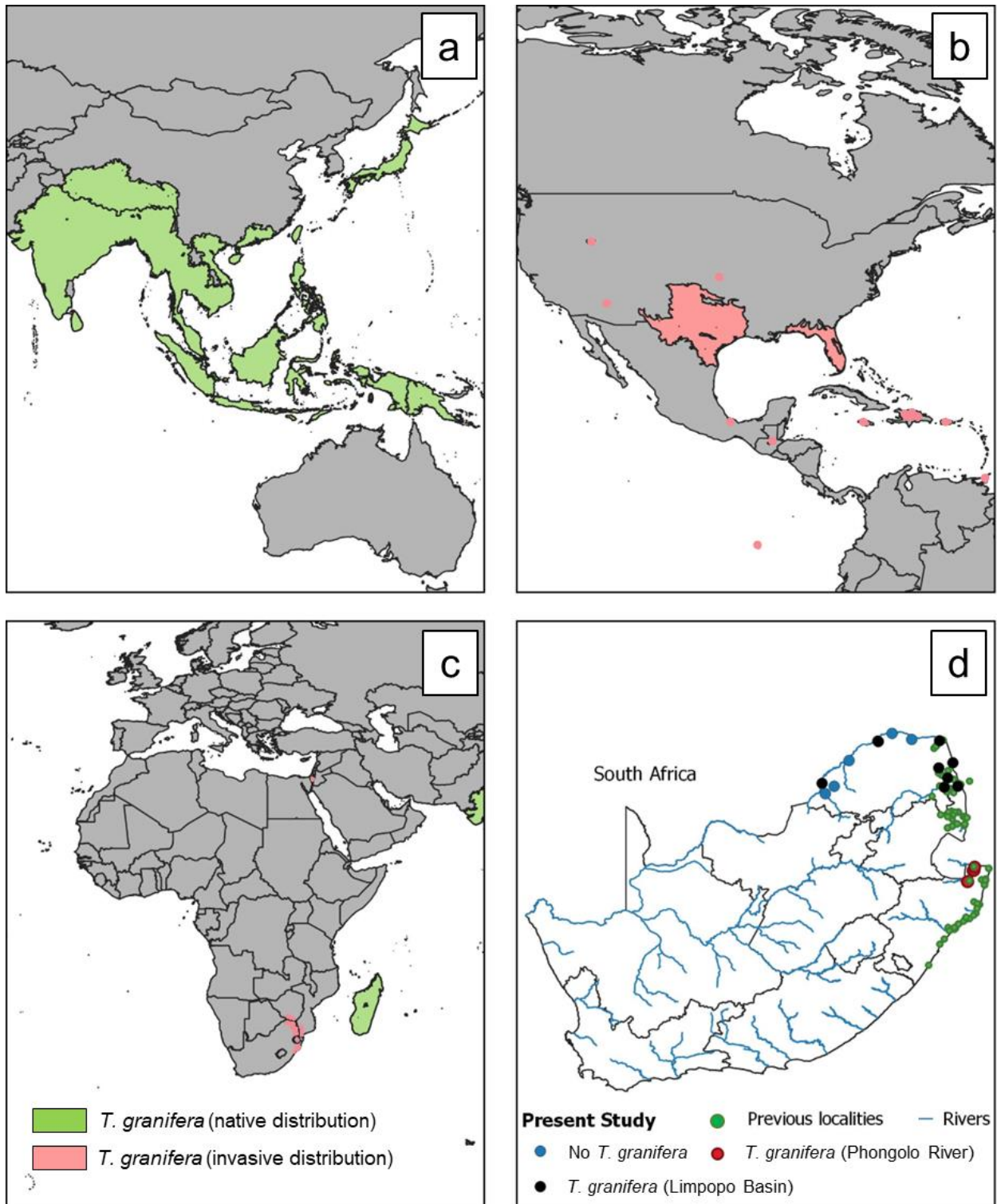


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1.2 Biology and reproduction

Tarebia granifera (Table 1.1) is primarily a benthic species and, in South Africa, has been collected on various substrates from natural and artificial aquatic habitats (Appleton *et al.*, 2009). Most localities invaded by *T. granifera* in South Africa lie below an altitude of 300 m.a.s.l. (Appleton *et al.*, 2009). While Abbott (1952) reported the snail from rivers at an altitude of 983 m on Guam Island, he noted that temperature was consistently higher than 24 °C, indicating that temperature plays a vital role in their distribution patterns. Miranda *et al.* (2010) indicated that *T. granifera* can survive temperature extremes of between 0 - 47.5 °C and may be able to survive cold fronts if the temperature stays above -1 °C. This is a wider temperature range than previously proposed in the literature (7 - 40 °C) by Chaniotis *et al.* (1980c). This ability may make it possible for *T. granifera* to invade areas at a higher altitude with colder temperatures, increasing the area they can invade in South Africa (Miranda *et al.*, 2010).

Table 1.1: *Tarebia granifera* taxonomic classification according to ITIS (2021).

Kingdom	Animalia
Phylum	Mollusca
Class	Gastropoda
Order	Neotaenioglossa
Family	Thiaridae
Genus	<i>Tarebia</i>
Species	<i>Tarebia granifera</i> (Lamarck, 1822)
Taxonomic status	"Valid"

Although *T. granifera* is considered a freshwater species the snail has also been found in estuarine environments, being reported in the St Lucia Estuary, South Africa (Miranda *et al.*, 2010). Experiments by Miranda *et al.* (2010) indicated that *T. granifera* could tolerate high salinity for several weeks. The snails can survive high salinity temporarily by reducing their physical activity, closing their operculum and also burying into sediment until freshwater returns and more favourable conditions are achieved (Chaniotis *et al.*, 1980a; Miranda *et al.*, 2010). The results from the aforementioned study found that lethal salinity for 50% of the population was reached at 30 psu (Practical Salinity Unit) between 65 to 75 days and 40 psu between 15 to 25 days. However, a "salinity shock experiment" indicated that the snails were unable to survive sudden increases in salinity (Miranda *et al.*, 2010). Although *T. granifera* is an aquatic species, it has been found to tolerate desiccation (air exposure) for two to three days at temperatures of 25 to 30 °C and 76 to 92% relative humidity (Chaniotis *et al.*, 1980c; Facon *et al.*, 2004; Miranda *et al.*, 2011b). Furthermore, *T. granifera* were reported to be tolerant of fast-flowing water due to their streamlined shape and ability to hold their position well during sudden velocity changes

(Sodeman, 1991). They have been found migrating upstream in streams discharging up to 0.05 m/s in the Caribbean islands (Prentice, 1983) and up to 1.2 m/s in KwaZulu-Natal, South Africa (Appleton *et al.*, 2009). Upstream migration was shown to be dependent on snail size, food distribution and habitat variety (Snider & Gilliam, 2008). The adaptations of this snail to tolerate temperature extremes, high salinity, desiccation, and fast-flowing water play a vital role in its success as an invader and significantly increase the aquatic ecosystems it will be able to invade (Wolmarans & de Kock, 2006; Appleton *et al.*, 2009; Miranda *et al.*, 2010; Jones *et al.*, 2017). Invaders with high ecological flexibility may reach higher population densities and inhabit larger regions, which play an essential role in their impact on the environment because these species can survive diverse environmental conditions and outcompete native biota (Nentwig *et al.*, 2010; Kesner & Kumschick, 2018).

Tarebia granifera are parthenogenetic (reproduction without fertilization) and ovoviviparous (eggs hatch within the parent), which is also key to its success as an invader (Appleton *et al.*, 2009). Shell length in adult snails may vary from 6 to 40 mm, but the most common size is about 25 mm (Abbott, 1952; Chaniotis *et al.*, 1980a). Sexual maturity is reached at a size between 5.5 - 8 mm within 122 days of age (Chaniotis *et al.*, 1980a; Prentice, 1983), although observations made by Appleton *et al.* (2009) indicated that although the first birth may occur in snails from 8 mm, most juveniles were born to snails bigger than 14 mm in size. New-born snails, 0.7 - 2.1 mm in size (Appleton *et al.*, 2009), emerge through a birth opening on the righthand side of the head (Abbott, 1952). These juveniles have a high survival rate (Chen, 2003), and can grow at a rate of about 0.16 mm/month (Chaniotis *et al.*, 1980a). *Tarebia granifera* can give birth to an average of one juvenile every 12 hours (Abbott, 1952; Chaniotis *et al.*, 1980a; Sodeman, 1991; Appleton *et al.*, 2009). The ability of an invader to reproduce rapidly and reach high abundances should correlate with their impacts (Parker *et al.*, 1999; Kesner & Kumschick, 2018). Invasive snail species have been reported to have higher probabilities of causing economic and environmental harm due to their ability to reproduce rapidly (Keller *et al.*, 2007; Rumi *et al.*, 2010; Nash & Hoffmann, 2012; Kesner & Kumschick, 2018).

Furthermore, *T. granifera* may also be able to spread from one waterbody to another through aquatic birds since small *T. granifera* (5 – 7 mm) have reportedly been found in unidentified bird droppings from the Mhlali River in KwaZulu-Natal (Appleton *et al.*, 2009). Birds seem to eat the snails and may pass them into another habitat (Appleton *et al.*, 2009). As some of the shells were still intact, the snails may have been alive when they passed the bird's digestive system and it is thought that they would perhaps have survived if they had been dropped in water (Appleton *et al.*, 2009). Hockey *et al.* (2005) reported *Melanoides tuberculata* (Müller, 1774), native to South Africa (Appleton, 2002), from the diet of the White-faced Duck, *Dendrocygna viduata* (Linnaeus, 1766).

According to Halse (1984), food is retained in the gut of aquatic birds for two to six hours and thus dispersal of snails (that survive in the gut) through birds is likely to occur as these birds move in short jumps from one aquatic ecosystem to the next (Appleton *et al.*, 2009). Dispersal may also occur via floating water plants, boats and water pumped from one waterbody to another, since *T. granifera* is known to be found on floating clumps of macrophytes that help make downstream dispersal more rapid and easy (Appleton *et al.*, 2009). Another mode of dispersal may be through drying mud on large mammals such as elephants (*Loxodonta africana* Blumenback, 1797) and buffalos (*Syncerus caffer* Sparrman, 1779), especially in conservation areas such as the Kruger National Park (Vanschoenwinkel *et al.*, 2008, 2011; van Leeuwen *et al.*, 2013). Alien species that are widely distributed have a higher probability of spreading and causing environmental changes in new areas (Moodley *et al.*, 2013; Novoa *et al.*, 2016; Kesner & Kumschick, 2018).

1.3 Mechanisms underlying impacts of *T. granifera*

The introduction of invasive species to aquatic ecosystems has become a key ecological factor, as invading organisms increasingly alter the native aquatic communities (Gurevitch & Padilla, 2004; Almeida *et al.*, 2018). Species that establish themselves outside of their native ranges usually achieve densities higher than those found in their native areas and potentially have negative impacts on native species as they alter the aquatic communities (Carlton *et al.*, 1990; Gurevitch & Padilla, 2004; Gallardo *et al.*, 2016). *Tarebia granifera* populations can reach very high densities, up to 10 000/m², and are often the dominant invertebrate species in invaded aquatic communities (Pointier *et al.*, 1998; Canete *et al.*, 2004; Pillay & Perissinotto, 2008; Appleton *et al.*, 2009). Appleton *et al.* (2009) reported an average density of 20 764/m² from the Nseleni River in KwaZulu-Natal, South Africa while Weyl *et al.* (2020) reported upon a snail community from the Phongolo River, indicating that 93% of the biomass consisted of *T. granifera*. It is therefore likely to have a negative impact on the indigenous benthos and community structures in invaded water bodies, resulting in a decline in the abundance of native species (Hillebrand *et al.*, 2002; Appleton *et al.*, 2009; Jones *et al.*, 2017; Miranda *et al.*, 2011a; Miranda & Perissinotto, 2014).

Native aquatic species may be particularly vulnerable to interference from dense populations of *T. granifera*, and studies on the ecological impact of this invader on aquatic community structures and biodiversity in South Africa are urgently needed (Appleton *et al.*, 2009; Weyl *et al.*, 2020). The effects that *T. granifera* may have on invertebrates and diatoms are also of concern as results from previous studies show that *T. granifera* can exploit large numbers of microphytobenthos and detritus, thus potentially causing top-down and cascading effects on the ecosystem (Miranda *et al.*, 2010; Miranda & Perissinotto, 2014). Benthic gastropods can cause sediment and microphytobenthos resuspension (Andersen, 2001; Orvain *et al.*, 2004), affecting the density of

invertebrates and their larval settlement (Jensen & Siegismund, 1980; Hunt *et al.*, 1987; Morrissey, 1988; Kelaher *et al.*, 2003). The high densities of this invader may indirectly limit energy transfers within food webs and can result in decreased benthic macroinvertebrate biodiversity (Miranda & Perissinotto, 2014; Hill *et al.*, 2015). The effects of *T. granifera* invasions on aquatic ecosystem biodiversity have been difficult to quantify but, in recent years, several studies have suggested that the establishment of *T. granifera* populations often results in decreased benthic biodiversity and disrupt aquatic environmental function (Miranda *et al.*, 2011a, 2011b; Moslemi *et al.*, 2012; Raw *et al.*, 2013; Miranda & Perissinotto, 2014).

The invasion success of *T. granifera* may be directly related to the release of chemical cues which drives away native gastropods (Raw *et al.*, 2013, 2015). Chemoreception in snails has been researched thoroughly, and snail species have become invasive species of global importance (Picker & Griffiths, 2011). Chemoreception enables organisms to respond to chemicals (with taste and smell), these chemicals act as signals for snails to respond in terms of movement (Wollerman *et al.*, 2003; Raw *et al.*, 2013, 2015). Previously it has been assumed that competition for food and space are the main mechanisms by which *T. granifera* displace native snail populations, but Raw *et al.* (2013) conducted a study that suggested otherwise. The aforementioned study found that three snails native to South Africa, *Assiminea* cf. *capensis* (Bartsch, 1915), *Coriandria durbanensis* (Tomlin, 1916) and *M. tuberculata* responded negatively (movement away from the source) due to chemical cues released by *T. granifera*. These chemical cues have the potential to impact native snail behaviour, causing them to vacate optimal habitat, reducing biotic resistance and thus facilitating invasion success (Raw *et al.*, 2013, 2015).

In another study, *T. granifera* was introduced in *Biomphalaria glabrata* (Say, 1818) habitats in the Dominican Republic and within 14 months results indicated that *T. granifera* was displacing *B. glabrata* (Perez *et al.*, 1991). Further, the authors also state that the displacement was not due to competition for food or space, instead *B. glabrata* avoids *T. granifera* possibly due to a chemical secreted by the invader. Although the responsible mechanism is not fully understood, this has led to intentional introductions of *T. granifera* and *M. tuberculata* as biocontrol agents for *Schistosoma* host snails in several countries, with profound impacts on schistosomiasis (also known as bilharzia) transmissions (Prentice, 1983; Perez *et al.*, 1991; Sodeman, 1991; Pointier & Jourdane, 2000; Appleton *et al.*, 2009; Pointier *et al.*, 2010; Hewitt & Willingham, 2019). *Tarebia granifera* was reported to cause local declines and extinctions in native snail populations from Cuba (Pointier & Jourdane, 2000; Karatayev *et al.*, 2009), Venezuela (Pointier & Giboda, 1999), Puerto Rico (Giboda *et al.*, 1997; Chaniotis *et al.*, 1980a, 1980b), and South Africa (de Kock & Wolmarans, 2008; Appleton *et al.*, 2009; Miranda *et al.*, 2011a, 2011b; Miranda & Perissinotto, 2012, 2014; Jones *et al.*, 2017; Kesner & Kumschick, 2018; Zengeya *et al.*, 2020). Native snails

such as *Chambardia wahlbergi* (Krauss, 1848), *Corbicula fluminalis* (Krauss, 1848), *Cleopatra ferruginea* (Lea, 1850), *Thiara amarula* (Linnaeus, 1758), *Lanistes ovum* (Troschel, 1845) and *M. tuberculata* are considered vulnerable (Appleton *et al.*, 2009).

When *T. granifera* is introduced in habitats occupied by *M. tuberculata*, it rapidly outnumbers the latter in terms of density (Pointier *et al.*, 1998). Miranda *et al.* (2011a) determined from previous reports and observations from South Africa that *M. tuberculata* and *T. amarula* in KwaZulu-Natal were less common while higher densities of the invasive *T. granifera* were recorded. As native snail species are being replaced by *T. granifera*, native predators such as shrimps (*Macrobrachium* sp. Spence Bate, 1868), crabs (*Potamonautes* sp. Macleay, 1838) and dragonflies (Libellulidae) may also be impacted as they are unable to feed on the invader due to their inability to break the harder shells of *T. granifera* (Appleton *et al.*, 2009; Miranda *et al.*, 2016). Further, streams in Tobago, Caribbean islands revealed an unusual habitat effect, where abundant empty shells from *T. granifera* washed downstream, providing a resource for hermit crabs (*Clibanarius tricolor* Gibbes, 1850 and *Clibanarius vittatus* Bosc, 1802) and drastically changed their patterns of shell use (Van Oosterhout *et al.*, 2013). The continued invasion and expansion of the *T. granifera* population in the island nation will result in significant long-term impacts on the hermit crab populations and demography (Van Oosterhout *et al.*, 2013). As for many island nations' wetlands, this invader represents a severe threat to South African invertebrate fauna (Kesner & Kumschick, 2018).

1.4 Feeding dynamics of *T. granifera*

Tarebia granifera feeds mainly on lower algae (mostly diatoms) and semi-decomposed organic food and their feeding impact can be much higher than that of other invertebrates (Miranda *et al.*, 2011a). Miranda *et al.* (2011a) reported that the snail can utilize up to 35% of available microphytobenthos (photosynthetic diatoms, cyanobacteria, flagellates, and green algae) per day and up to 68% of the daily primary benthic production. They can significantly reduce algal standing stocks, and similar values to those given by Miranda *et al.* (2011a) have been reported for zooplankton species, known for affecting ecosystem function (Perissinotto, 1992; Kibirige & Perissinotto, 2003). According to Miranda *et al.* (2011a), the ingestion rate of *T. granifera* is comparable to that of the golden apple snail (*Pomacea canaliculata* Lamarck, 1822), listed as one of the world's worst invasive species (Lowe *et al.*, 2000; Baker *et al.*, 2010). The generalist diet of *T. granifera* is yet another characteristic of this invader that may have contributed to its successful establishment in southern Africa (Miranda & Perissinotto, 2012). As invasive species disrupt trophic functioning, native species tend to shift their dietary preference (Vander Zanden *et al.*, 1999). This was evident in coastal lakes from KwaZulu-Natal in South Africa, where the native

Assiminea ovata (Krauss, 1848) started to feed less on microphytobenthos and more on branching algae after an increase in abundance of *T. granifera* (Miranda & Perissinotto, 2012).

Studies on temperate and tropical ecosystems have found that invasive snails can alter nutrient cycles in freshwater systems (Arnott & Vanni, 1996; Hall *et al.*, 2003; Carlsson *et al.*, 2004). A study conducted by Moslemi *et al.* (2012) indicated that riparian cover influenced the effect that *T. granifera* had on nitrogen (N) cycling in the West Indies. This study found that snail densities were up to eight times higher and N excretion up to nine times higher in open-canopy habitats than in habitats covered by riparian vegetation. Changes in the availability of nutrients influence the number of nutrients that limit primary production, and thus the growth and community structures of primary producers, e.g., plants and algae (Vanni, 2002). The snail's impact on available resources may alter food web dynamics, with unknown implications on aquatic consumers and higher trophic levels (Moslemi *et al.*, 2012).

1.5 Disease transmission from *T. granifera*

Thiarid snails (*T. granifera* and *M. tuberculata*) pose a severe threat to public health as they transmit parasites of fishes, birds, and mammals (Sodeman, 1991; Mitchell *et al.*, 2000; Veeravechsukij *et al.*, 2018a). These snails act as the first intermediate hosts for trematodes that have been reported to affect the respiratory, intestinal, and hepatic systems of not only wildlife but humans as well (Sodeman, 1991; McKoy *et al.*, 2011; Veeravechsukij *et al.*, 2018a). *Tarebia granifera* are well known to harbour diverse and prevalent species of trematodes both within and outside their native range, such as *Haplorchis pumilio* Looss, 1896 (Veeravechsukij *et al.*, 2018a), *Haplorchis taichui* Nishigori, 1924 (Ditrich *et al.*, 1992; Chontanarith & Wongsawad, 2010, 2013; Le *et al.*, 2017; Veeravechsukij *et al.*, 2018a), *Loxogenoides bicolor* Kaw, 1945 (Dechruksa *et al.*, 2007; Veeravechsukij *et al.*, 2018a), *Centrocestus formosanus* Nishigori, 1924 (Dechruksa *et al.*, 2007; Veeravechsukij *et al.*, 2018a), *Acanthatrium histaense* Koga, 1953 (Dechruksa *et al.*, 2007; Veeravechsukij *et al.*, 2018a), *Haematoloechus similis* Looss, 1899 (Grabda, 1960; Dechruksa *et al.*, 2007), *Transversotrema laruei* Velasquez, 1958 (Veeravechsukij *et al.*, 2018a), *Cardicola alseae* Meade and Pratt, 1965 (Ukong *et al.*, 2007; Veeravechsukij *et al.*, 2018a), *Alaria mustelae* Bosma, 1931 (Bosma, 1934; Veeravechsukij *et al.*, 2018a) and *Philophthalmus gralli* Mathis and Léger, 1910 (Tolley-Jordan & Owen, 2008; Heneberg *et al.*, 2014; Veeravechsukij *et al.*, 2018a). To date, no such trematodes have been reported in *T. granifera* from South Africa (Miranda *et al.*, 2011b; Weyl *et al.*, 2020).

Biological invaders may have an advantage over native species if they have immunity against enemies such as predators, pathogens or parasites within invaded habitats or if such enemies are missing from invaded habitats (Genner *et al.*, 2008). Field evidence from Lake Malawi

indicated that trematodes were absent from a highly invasive morph of *M. tuberculata*, and that the invader was resistant to native trematodes found in the native *M. tuberculata* (Genner *et al.*, 2008). Parasites may not have migrated with the host or were unable to survive in the invaded habitat due to the absence of suitable intermediate or final hosts (Keane & Crawley, 2002; Torchin *et al.*, 2003; Genner *et al.*, 2008). Another reason may be that the invasive snail has immunity to native parasites as native parasites may be unable to infect invading taxa due to their evolutionary adaptations to native host species (Ebert, 1994, 1998; Prenter *et al.*, 2004; Fromme & Dybdahl, 2006). Trematodes play a vital role in host snail populations as they can affect changes in survivorship, behaviour and morphology, as well as cause reduced fecundity and growth (Sorensen & Minchella, 2001; Lafferty & Kuris, 2009; McKoy *et al.*, 2011). Genner *et al.* (2008) report that native trematodes act as parasitic castrators and cause reduced population growth within the native snail communities, this may give invasive snails an advantage in terms of reproduction and contribute to rapid population expansion. These changes can have considerable effects on the population dynamics and competitive interactions among invaders and native species (Byers & Goldwasser, 2001; Genner *et al.*, 2008).

Tarebia granifera has been reported to serve as a host for invasive parasites within invaded habitats in Jamaica (McKoy *et al.*, 2011) and Texas (Tolley-Jordan & Owen, 2008). From Jamaica, *T. granifera* has been found to be infected with two species of invasive trematodes: a *Notocotylus* sp. and a *Philophthalmus* sp. (oriental avian eye fluke). This was also the first report of a *Notocotylus* sp. in *T. granifera* and a *Philophthalmus* sp. occurring in Jamaica (McKoy *et al.*, 2011). Tolley-Jordan and Owen (2008) also found invasive oriental avian eye flukes (*Philophthalmus gralli* Mathis & Leger, 1910) infecting *T. granifera* in Texas. Wading birds are known to be definitive hosts for notocotylids (Skirnisson *et al.*, 2004) and many species of birds, including birds of the orders Galliformes (ground-feeding birds) and Anseriformes (waterfowl), harbour philophthalmids (Murray, 1964; Nollen & Murray, 1978). Humans have been reported as incidental hosts for philophthalmids in Yugoslavia, Sri Lanka, Thailand, Mexico, and the United States (Rajapakse *et al.*, 2009), these infections cause swelling of the semilunar fold in the eyes (Mimori *et al.*, 1982). The trematodes *H. pumilio* and *H. taichui* are widely distributed, reported from at least 14 countries worldwide (Ditrich *et al.*, 1992; Huston *et al.*, 2014; Veeravechskij *et al.*, 2018a), and are known to infect both *T. granifera* (Chontanarith & Wongsawad, 2010, 2013; Le *et al.*, 2017; Veeravechskij *et al.*, 2018a) and *M. tuberculata* (Tolley-Jordan & Owen, 2008; Krailas *et al.*, 2011, 2014; Huston *et al.*, 2014; Lopes *et al.*, 2020; Pulido-Murillo *et al.*, 2018). Invasion by these parasites can have severe pathological consequences for a broad range of intermediate fish hosts (Huston *et al.*, 2014) since the penetration and migration of large numbers of cercaria can be lethal to fry and adult fish species (Sommerville, 1982a; Umadevi & Madhavi,

2006). Natural definitive hosts are fish-eating animals, including humans (Chai *et al.*, 2009; Giboda *et al.*, 1991a).

Even though no parasites have been reported from *T. granifera* in South Africa, the native thiarid *M. tuberculata* was previously reported to be infected with at least nine unidentified cercariae (Faust, 1921; Porter, 1938), which have been described under the generic collective group *Cercaria* (Müller, 1773). Unfortunately, the last reports describing parasites from *M. tuberculata* in South Africa were in fact, in 1921 by Faust and in 1938 by Porter. Due to a lack of research in recent years and the fact that *M. tuberculata* is known to be a host for diverse groups of trematodes worldwide (Pinto & de Melo, 2011), it is estimated that the trematode diversity from this snail species might be higher than expected. Additionally, molecular work on cercaria may make the process of identification much easier than in the past. As previously mentioned, *T. granifera* reach very high densities within invaded habitats and have shown to replace *M. tuberculata* as well as several other native snail species. Based on the many possible ways invasive and native host parasite species can interact, it might be possible that *T. granifera* can a) dilute native parasite populations via host displacement, b) serve as a host to invasive parasites that are brought in via migratory birds, or c) eventually serve as a host for native parasite taxa that spillover from native hosts (Chalkowski *et al.*, 2018). Consequently, studies on the parasitic diversity of both the thiarid species in South Africa will be of great significance and will contribute to a better understanding of the biodiversity, biology and evolutionary relationships of these snail hosts and their trematodes.

1.6 *Tarebia granifera* as prey

The successful establishment of invasive species can be affected by predation from native species (MacNeil *et al.*, 2013). The higher competitive ability and successful invasion of alien species can be explained by the enemy release hypothesis, e.g., resistance to predation, parasites, and pathogens (Elton, 1958; Tilman, 1999). The invasive *T. granifera* can be preyed upon by birds, fish, and invertebrates, however, it does not seem to have many successful predators in South Africa (Miranda & Perissinotto, 2012; Miranda *et al.*, 2016). Mere examples of native species preying on invaders do not demonstrate that predation limits the establishment, spread or abundance of the invader (MacNeil *et al.*, 2013). To understand how predation may influence the invasion process, evidence of negative interactions between native predators and invasive species is required (MacNeil *et al.*, 2013; Miranda *et al.*, 2016). The crab *Potamonautes sidneyi* (Rathbun, 1904), native to South Africa, has been reported to prey upon *T. granifera* in Lake Sibaya, South Africa (Peer *et al.*, 2015). However, Miranda *et al.* (2016) reported that two native freshwater crabs: *Potamonautes perlatus* (Milne Edwards, 1837) and *P. sidneyi* were unable to feed successfully on *T. granifera* due to their more rigid shells compared to the native

M. tuberculata. Miranda *et al.* (2016) reported that some of the large male crabs were able to crush the shells of *T. granifera*, but the overall successful attacks on the invasive snail were much lower than those on the native *M. tuberculata*. The study found that the crabs had a higher difficulty in handling *T. granifera* and certain individuals had no success in crushing their harder shells (Miranda *et al.*, 2016). The invasive snail is less vulnerable to predation than native snail species, thus further facilitating their establishment and spread in South Africa (Miranda & Perissinotto, 2012; Miranda *et al.*, 2016). When alien prey species are harder to feed upon, predators will continue to prefer native species, which also have to compete with the invader (Shinen *et al.*, 2009; López *et al.*, 2010).

Predators often eat prey that is most abundant when they hunt (known as frequency-dependent predation), predators often switch between prey as prey species abundance changes over time (Allen, 1988; Elliott, 2004; Morrison *et al.*, 2019). Other factors which also influence a predator's preference for a certain prey include energy gained from the prey relative to the energy cost of catching the prey, e.g., handling a prey with a hard shell and time required to search for prey (Morrison *et al.*, 2019). Native predators may quickly incorporate invasive species into their diet if they can easily prey upon them, thus potentially increasing biotic resistance towards alien species (López *et al.*, 2010; Barrios-O'Neill *et al.*, 2015). Morrison *et al.* (2019) suggest that the Eastern musk turtle *Sternotherus odoratus* (Latreille, 1802) in Texas may have shifted its diet towards invasive snails due to a three-decade presence and high abundance of the invasive snails. The aforementioned study found that the invasive *M. tuberculata*, *T. granifera*, and the Giant Ramshorn Snail *Marisa cornuarietis* (Linnaeus, 1758) were the most abundant species identified from *S. odoratus* faecal samples. *Tarebia granifera* were found in half the samples investigated during the study (Morrison *et al.*, 2019). Additionally, there are numerous reports of native turtles preying on invasive species (Ford & Moll, 2004; Yusa *et al.*, 2006; Bulte & Blouin-Demers, 2008; Patterson & Lindeman, 2009; Wilhelm & Plummer, 2012; Atkinson, 2013). Predators may become sufficient at utilising invasive prey through natural selection over time, especially if the prey becomes abundant and provides large amounts of energy relative to cost (Morrison *et al.*, 2019).

1.7 Interactions with other invasive species

Ecosystems can become invaded by multiple invasive species, and these invasions lead to interactions that could enhance the negative impacts on native communities and their ecosystems (Johnson *et al.*, 2009; Makherana *et al.*, 2022b). A mesocosm study in the U.S.A. on the invasive rusty crayfish, *Orconectes rusticus* (Girard, 1852), and Chinese mystery snails, *Cipangopaludina chinensis* (Gray, 1834), revealed that the two invaders had weak negative impacts on one another, but both negatively affected native snail biomass and abundance (Johnson *et al.*, 2009).

The aforementioned study found that the two invasives drove one native snail to extinction and reduced a second species by more than 95%. The crayfish (a snail predator) had significantly higher impacts on native snails compared to the invasive snail due to the thicker shell and larger size of the invader (Johnson *et al.*, 2009).

Three invasive snail species (*Corbicula fluminea* (Müller, 1774), *M. tuberculata* and *T. granifera*) from the Tuxpan and Tecolutla Rivers in Mexico have been reported to have serious impacts on native snail abundances due to the difference in distribution patterns of these invaders (López-López *et al.*, 2009). Native snail species have been confined to narrow habitats and occur in low densities, *T. granifera* and *C. fluminea* indicated strong dominance, while *M. tuberculata* and native snails were reported to have limited distribution. López-López *et al.* (2009) indicated that *T. granifera* reached higher population densities, likely due to competition with the other species and its ability to rapidly recolonize ecosystems after pronounced depletions in snail density during the heavy rainy season. *Tarebia granifera* populations responded quickly during the dry season when nutrients increased food availability, and in these circumstances, were more successful than both the other invasive and native snails (López-López *et al.*, 2009). A study from the state of Texas (U.S.A.) suggested that the negative interactions between native and invasive species may be due to differences in habitat use rather than competition (Tolley-Jordan & Owen, 2008). The authors found that the two invasive snails (*M. tuberculata* and *T. granifera*) co-occurred in lentic habitats while *M. tuberculata* and the native *Elimia comalensis* (Pilsbry, 1890) did not co-occur. In lentic habitats, the invaders had similar densities and even though *E. comalensis* had the highest densities of all four native species, it was still 200 times less than the densities of the invaders (Tolley-Jordan & Owen, 2008). *Tarebia granifera* and *E. comalensis* distributions positively co-occurred in lotic habitats, possibly due to *T. granifera* being able to tolerate faster water flow than *M. tuberculata* (Dussart & Pointier, 1999) and the preference of *E. comalensis* for lotic habitats (Tolley-Jordan & Owen, 2008).

Intensely competitive interactions occur among exotic species with similar ecologies, for instance, the invasion of *T. granifera* and a new morph of *M. tuberculata* (MA strain) seriously impacted other morphs of *M. tuberculata* in Guadeloupe and Martinique, Caribbean islands (Pointier *et al.*, 2010). These invasions led to the extinction of one morph of *M. tuberculata* (FDF strain) while others became scarce. Another example from the Great African Lakes indicated that the invasion of Asian morphs of *M. tuberculata* threatens native thiarid species diversity (Genner *et al.*, 2004). Where endemic thiarid diversity exists, similar threats can be expected (Pointier *et al.*, 2005; Simone, 2006; Pointier *et al.*, 2010). Although *M. tuberculata* is native to Africa, invasive morphs may be present in South Africa due to the complex global invasion history of the snail (Genner *et al.*, 2004; Van Bocxlaer *et al.*, 2015; Miranda *et al.*, 2016). Where *T. granifera* and *M. tuberculata*

are found in the same habitat, the interactions between the two species result in mutual exclusion (Pointier *et al.*, 1998; Contreras-Arquieta & Contreras-Balderas, 1999; Karatayev *et al.*, 2009; López-López *et al.*, 2009).

Furthermore, invasive snail populations should not be regarded as genetically depauperate (Pointier *et al.*, 2010). Introductions of *M. tuberculata* in Martinique and Guadeloupe gave rise to rare sexual events between introduced morphs forming natural hybrids, and these hybrids have high levels of genetic variation and ecologically important traits (Facon *et al.*, 2008). Different in morphology and life history from their parents, these hybrids have surpassed them (Facon *et al.*, 2005). These findings call attention to the importance of understanding interactions among invasive species, as they can have severe effects on ecosystem function and community structures (Johnson *et al.*, 2009). Further research on this subject is needed to better understand these interactions (Pointier *et al.*, 2010).

1.8 Control measures

For complete eradication of *T. granifera* from an environment, the entire population needs to be eliminated due to its parthenogenic abilities (Miranda *et al.*, 2010). The use of molluscicides in the natural environment will not be a practical method of control as non-target species may be adversely affected (McCullough *et al.*, 1980; Miranda *et al.*, 2010). A 'salinity shock experiment' by Miranda *et al.* (2010) indicated that *T. granifera* populations were at great risk when water salinity was suddenly increased. This led to suggestions that the invader could be controlled in temporarily open/closed estuaries by artificially breaching the mouth (Miranda *et al.*, 2010). However, the manipulation of natural environments on such a scale may have unintended and negative consequences on these ecosystems (Miranda *et al.*, 2010).

The black carp *Mylopharyngodon piceus* (Richardson, 1846), a freshwater snail predator, has been effectively used as a biological control of *M. tuberculata* and *T. granifera* in several parts of the world (Ben-Ami & Heller, 2001; Hung *et al.*, 2013; Yakovenko *et al.*, 2018). The assassin snail *Anentome helena* (von dem Busch, 1847) has also been reported to be an effective biological control in Malaysia, Indonesia, Thailand, and Laos (Schiffbauer, 2009; Yakovenko *et al.*, 2018). However, the introduction of a biological control has numerous environmental risks that need to be considered, and extensive research needs to be done before attempting to use such agents in new environments (Howarth, 1991; Simberloff & Stiling, 1996; Hoddle, 2004; Louda & Stiling, 2004). As previously mentioned, trematodes play a vital role in the natural control of snail populations due to their ability to affect changes in snail survivorship, behaviour, morphology and cause reduced fecundity and growth (Sorensen & Minchella, 2001; Genner *et al.*, 2008; Lafferty & Kuris, 2009; McKoy *et al.*, 2011). In a study conducted by McKoy *et al.* (2011), only 3% of *T.*

granifera infected with trematodes had reproductive stages (eggs, embryos, or juveniles), compared to 90% of snails that were not infected. Though potentially significant, the ecological impact of such trematodes on the fecundity of *T. granifera* and the environment needs to be investigated further (McKoy *et al.*, 2011). Unfortunately, the control of *T. granifera* within the natural environment may have severe effects on the environment and native biota, it therefore, seems to be a nearly impossible task. Likewise, due to a lack of management (Makherana *et al.*, 2022a) and the wide area invaded by *T. granifera* within the eastern and northern parts of southern Africa (Appleton *et al.*, 2009), this menace will probably continue in the future (Kesner & Kumschick, 2018).

Apart from their environmental impacts and their role as an intermediate host for medically and economically important trematodes, *T. granifera* has been reported to pose a risk to important infrastructure (Appleton *et al.*, 2009; Yakovenko *et al.*, 2018; Oleh *et al.*, 2018). The snail reportedly colonised reservoirs of three large industrial plants, blocking pipes and damaging equipment, it also interfered with water circulation at a fish hatchery in northern KwaZulu-Natal, South Africa (Appleton *et al.*, 2009). In addition, *T. granifera* and *M. tuberculata* have recently been reported to pose a threat to the hydraulic cooling structures in the Zaporizhia Nuclear Power Plant, Ukraine (Yakovenko *et al.*, 2018; Oleh *et al.*, 2018). This has led to laboratory investigations on the biological and biomechanical methods to control these snails.

From the Zaporizhia Nuclear Power Plant, Ukraine, Yakovenko *et al.* (2018) found that the thiarid snails were unequally distributed with an average abundance of 3.3 *T. granifera* to one *M. tuberculata*. Experimental trials on marbled crayfish *Procambarus virginalis* (Lyko, 2017), pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) and *Botia lohachata* (Chaudhuri, 1912) were found to be unsuccessful in snail control. The assassin snail was noted to be a successful control agent however, they found that when the predatory snail has more accessible feeds (organic matter), the snail consumes smaller quantities of the thiarid snails (Oleh *et al.*, 2018). According to Oleh *et al.* (2018), the most successful method to control *T. granifera* populations from the power plant was through the use of snail traps, designed to attract the snails with bait. Snails would fall into the traps as they moved towards the bait and were unable to escape the traps (Oleh *et al.*, 2018). The authors found that these traps also made it possible to catch sexually mature snails, thus removing reproductive individuals from the population. In addition to the use of snail traps as proposed by Oleh *et al.* (2018), Yakovenko *et al.* (2018) suggested that the power plant should stock their cooling ponds with the molluscivorous species *M. piceus* (black carp) and *A. helena* (assassin snails). This research may aid in the development of control measures for reducing the negative impact of accidental introductions of snails into technical reservoirs of strategic importance (Yakovenko *et al.*, 2018; Oleh *et al.*, 2018). As the effective control of

established *T. granifera* populations in a natural setting remains challenging, the most effective method to prevent snail invasions in the future will be to implement strict policies that regulate the global trade of such species (Preston *et al.*, 2022).

1.9 Problem statement

Management and remediation of biological invasions in South Africa are in need of a more complete understanding of this invader's impact and the mechanisms through which they occur (Burlakova *et al.*, 2010; Miranda *et al.*, 2011b; Weyl *et al.*, 2020; Makherana *et al.*, 2022a). Previous studies have investigated the impacts and population structures of *T. granifera* in coastal lakes and estuaries in KwaZulu-Natal (see Miranda *et al.*, 2010, 2011a, 2011b; Miranda & Perissinotto, 2014). However, less is known regarding the current distribution, population dynamics and impacts *T. granifera* may have on the native biodiversity and aquatic community structures from freshwater ecosystems in South Africa (Makherana *et al.*, 2022a). Especially since the current distribution and densities of native, as well as invasive, freshwater snail species have been underreported in recent years, particularly in the conservation areas of Mpumalanga and Limpopo. Furthermore, this invader may also act as a potential intermediate host for parasitic trematodes (native and/or invasive parasites) in South Africa, which might be infecting fish, birds, mammals and potentially humans. Consequently, there is a need to investigate the potential impacts of *T. granifera* on native aquatic ecosystems and to investigate the role of this invader as a host for disease-causing trematodes infection in South Africa.

1.10 Aims, objectives and hypotheses

Study aims:

1. Investigate the current distribution, densities, and population size structures of *T. granifera* at selected sites on the Limpopo River system and Phongolo River of southern Africa.
2. Determine the impacts of *T. granifera* on the aquatic community structures of benthic diatoms at selected *in situ* sites on the Limpopo River system and Phongolo River.
3. Corroborate the findings of the *in situ* evaluation of the impacts of *T. granifera* on benthic diatoms with an experimental microcosm study.
4. Investigate the role of the invasive thiarid (*T. granifera*) versus the native thiarid (*M. tuberculata*) as hosts for digenean trematodes within the Limpopo River system and Phongolo River.
5. Determine the genetic diversity of *T. granifera* from the selected sites on the Limpopo River system and Phongolo River.

Study objectives:

1. Determine the current distribution, densities, and population size structures of *T. granifera* at selected sites on the Limpopo River system and Phongolo River in southern Africa.
2. Quantitatively sample, identify and count diatoms from selected sites of the Phongolo River and Limpopo River system where different *T. granifera* population densities were sampled.
3. Describe the effects of *T. granifera* on benthic diatom community structures using univariate and multivariate statistical methods based on *T. granifera* density.
4. Use experimental microcosm studies to describe the ecological impacts of *T. granifera* on benthic diatoms in a controlled environment.
5. Investigate the possible parasitic infection of *T. granifera* and *M. tuberculata* with cercariae shedding experiments, with snail dissections and molecular analysis.
6. Make use of molecular analysis to identify any parasitic species within the two aforementioned snail species to the lowest possible taxonomic position.
7. Investigate the genetic diversity of *T. granifera* from selected sites on the Limpopo River system and Phongolo River with the use of molecular and phylogenetic analyses.

Hypotheses:

1. *Tarebia granifera* will occur at densities greater than within their native range and population structures will reflect an established population with an even spread of individuals throughout the size classes.
2. *Tarebia granifera* density are related to water quality parameters such as pH, conductivity and temperature.
3. High densities of invasive *T. granifera* is expected to correlate with low native mollusc densities and diversity, suggesting that the invader can displace native mollusc communities.
4. High densities of *T. granifera* in an aquatic community will result in a decrease of benthic diatom diversity, disrupting benthic diatom community structures and functionality.
5. *Tarebia granifera* acts as a first intermediate host for medically and ecologically important trematodes within the invaded ecosystems sampled, which may spillover to native species.

6. Due to the absence of males and the parthenogenic reproduction of *T. granifera*, the snails will have a low genetic diversity in South Africa.

1.11 The layout of this dissertation

Chapter 1: Provides background information, and a review of the available literature regarding *T. granifera* on a global scale as well as in South Africa, the aims, objectives and hypotheses of the study are also provided.

Chapter 2: Contains information on the selected study areas and detailed site descriptions of all the sites sampled on the Limpopo River system and Phongolo River.

Chapter 3: Focusses on the current distributions, densities and population size structures of *T. granifera* as well as a brief overview of the native snail diversity and subsequent effects of *T. granifera* on this diversity from the selected sites on the Phongolo River and Limpopo River system.

Chapter 4: An *in situ* ecological assessment of changes in benthic diatom diversity and community structures in relation to *T. granifera* population densities from selected sites on the Limpopo River system and Phongolo River.

Chapter 5: Presents an experimental microcosm study to determine the effects of *T. granifera* on the aquatic community structures of benthic diatoms in a controlled environment.

Chapter 6: Investigate the role of the two thiarid snail species, the invasive *T. granifera* versus the native *M. tuberculata*, as hosts for parasitic trematodes in the Limpopo River system and Phongolo River, as well as an investigation on the genetic diversity of *T. granifera* with the use of molecular and phylogenetic analysis.

Chapter 7: Provides concluding remarks on the main research findings in relation to the study aims and hypotheses, it also highlights the study limitations and provide recommendations for future research.

Chapter 2: Study area

2.1 Introduction to the study regions

For this study a total of 19 sites were selected for sampling (Figure 2.1). Sites were selected based on known distributions of aquatic snails (invasive and native) as well as accessibility. Four of the selected and surveyed sites were on the Phongolo River in northern KwaZulu-Natal, sampled in 2017. A total of 15 sites were sampled on the Limpopo River and its tributaries (Limpopo River system), as part of the Limpopo Watercourse Commission (LIMCOM) 2021 High Flow Survey. Site coordinates and other relevant site-related information are listed in Table 2.1.

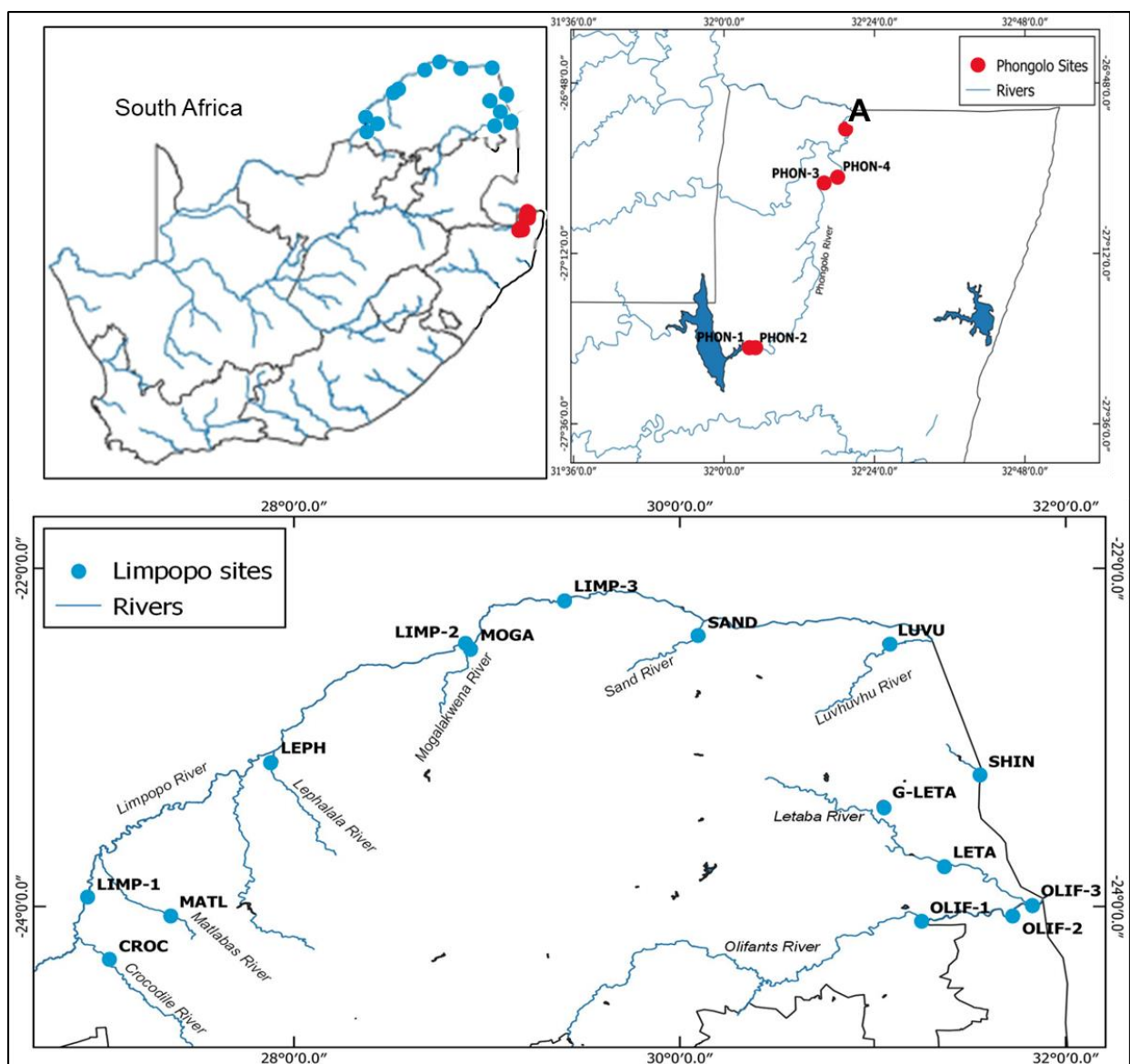


Figure 2.1: Map indicating all selected sites on the Limpopo River system (blue points) and Phongolo River system (red points), the microcosm study placement is indicated by point A.

Table 2.1: Site names, sampling dates, location, coordinates and discharge data for sampling sites on the lower Phongolo and Limpopo River systems sampled during this study. Discharge values in “Bold” were average daily flow measured by the Department of Water and Sanitation gauging stations located closest to the relevant site.

Site Code	Sampling Date	River	Coordinates	Discharge (m ³ /s)	Elevation (m.a.s.l.)	FBIS/ Descriptive Site Code
PHON-1	31/08/2017	Phongolo	-27.421003, 32.067122	N/A	~140	Pongolapoort Dam
PHON-2	31/08/2017	Phongolo	-27.42268, 32.08153	1.821	~73	Below the dam wall
PHON-3	31/08/2017	Phongolo	-27.03677, 32.26647	1.821	~33	Bridge
PHON-4	02/09/2017	Phongolo	-27.02063, 32.30313	1.913	~32	Walkway
A	07/10/2017	Phongolo Floodplain	-26.908034, 32.324021	N/A	~26	Microcosm placement
CROC	21/04/2021	Crocodile	-24.31417, 27.04614	7.212	885	A2CROC-ROOIB
LIMP-1	22/04/2021	Limpopo	-23.9485, 26.93123	6.586	857	A4LIMP-SPANW
MATL	23/04/2021	Matlabas	-24.05186, 27.35964	0.181	916	A4MATL-PHOFU
LEPH	24/04/2021	Lephalala	-23.14128, 27.88503	3.506	794	A5LEPH-SEEKO
LIMP-2	25/04/2021	Limpopo	-22.45519, 28.90175	10.038	631	A6LIMP-LIMPK
MOGA	26/04/2021	Mogalakwena	-22.47344, 28.91950	0.000	636	A6MOGA-LINPK
LIMP-3	27/04/2021	Limpopo	-22.18383, 29.40519	9.739	511	A7LIMP-MAPUN
SAND	28/04/2021	Sand	-22.39928, 30.09942	0.009	447	A7SAND-R508B
LUVU	29/04/2021	Luvuvhu	-22.44444, 31.08344	17.433	249	A9LUVU-MUTAL
OLIF-1	30/09/2020 05/05/2021	Olifants	-24.08642, 31.25094	1.692 9.862	278	B7OLIF-MAMB1
OLIF-2	04/10/2020 03/05/2021	Olifants	-24.05214, 31.72878	2.537 10.670	185	B7OLIF-BALUL
OLIF-3	03/10/2020	Olifants	-23.99071, 31.82659	5.364	130	B7OLIF-LETAB
G-LETA	06/05/2021	Great Letaba	-23.677528, 31.098639	1.788	318	B8GLET-IFR16
LETA	04/05/2021	Letaba	-23.943389, 31.735111	1.656	264	B8LETA-NGWEN
SHIN	01/05/2021	Shingwedzi	-23.22194, 31.55492	0.012	241	B9SHIN-POACH

2.1.1.1 The Phongolo River system

The Phongolo River rises near Wakkerstroom (Mpumalanga Province, South Africa), flowing east towards the Lebombo Mountains (KwaZulu-Natal) and into the Pongolapoort Dam (Lankford *et al.*, 2011). The Phongolo River Floodplain lies downstream of the dam all the way up to the junction of the Phongolo- and Usuthu Rivers. The floodplain is South Africa's largest (13000 ha when fully inundated) and most diverse floodplain ecosystem, with ~50 fish species, > 40 frog species, and > 430 bird species (SAWCP, 1996; Smit *et al.*, 2016; Acosta *et al.*, 2020). The floodplain has an area of 120 km² and approximately 90 floodplain depressions (commonly referred to as pans in South Africa), of which all are ecologically and economically important (Lankford *et al.*, 2011; Dube *et al.*, 2015, 2020; de Necker *et al.*, 2020, 2022). The climate consists of subtropical (hot and humid) conditions, where temperatures can reach well over 40 °C with an average seasonal summer rainfall of 634 mm from December to March (Kyle & Marneweck, 1996; Lankford *et al.*, 2011). The Ndumo Game Reserve, located at the junction of the Usuthu- and Phongolo River systems, is the only protected area (10 117 ha) found within the floodplain (Kyle & Marneweck, 1996). The Ndumo Game Reserve was also placed on the list of Wetlands of International Importance, under the Ramsar convention in 1997, due to the wide range of unique wetlands and biodiversity (Kyle & Marneweck, 1996). The Phongolo system is dammed (Pongolapoort Dam) directly upstream of the floodplain, mismanagement and untimely flows have resulted in a disconnection between the river and its floodplain, as it is only flooded after controlled water is released from the dam, when it nears full capacity (Lankford *et al.*, 2011; Dube *et al.*, 2015; Welicky *et al.*, 2017; de Necker *et al.*, 2020). The disconnection from the river has been exacerbated due to suprasedseasonal droughts in the area and as a result, no controlled flood releases have occurred since 2014 (de Necker *et al.*, 2020). The lack of water flow has resulted in almost total desiccation of the floodplain, with only two of the approximately 90 floodplain pans containing water (de Necker *et al.*, 2020). The Phongolo River was selected as it is known to have *T. granifera* (Smit *et al.*, 2016; Dube *et al.*, 2017, 2019). Sampling sites were selected within the Phongolo River system, stretching downstream from the Pongolapoort Dam to just before the confluence with the Usuthu River inside the Ndumo Game Reserve. Site selection was based on study sites used as part of previous studies in the region (Dube *et al.*, 2017; de Necker, 2016, 2019; de Necker *et al.*, 2020, 2022).

The first site (PHON-1) is located on Pongolapoort Dam at Tiger Lodge (Figure 2.2a-b). During the survey, the water had a slight brown colour with patches of algae. The weather consisted of sunny and warm conditions. The marginal vegetation was dominated by grasses and some herbaceous plants and sedges. The substrate was made up of gravel, sand and mud. The second site (PHON-2) is immediately below the dam wall (Figure 2.2c-d), approximately 5 km

downstream from the first site. Water flow was visually rated low, algae were dominant, and water had a brown-green tint. The weather consisted of sunny and warm conditions. Reeds dominated the marginal vegetation, with a few types of grasses present. Water was mostly shallow, and sand-mud dominated the substrate. Instream habitat was rated low in diversity. The third site (PHON-3) is located approximately 50 km further downstream from PHON-2 at a high-water bridge (Figure 2.2e-f). During the survey, water was green in colour with low flow conditions. Sunny and warm weather was noted. The marginal vegetation consisted of shrubs, grasses, reeds and herbaceous plants. Hydraulic biotopes consisted of deep and shallow runs. Sand, mud and fine gravel dominated the substrate. The instream habitat was rated moderate to low in diversity. Rubbish was present along the banks of the river, most likely from a village located close to the site. Local people at the site were also collecting water for household use. The most downstream site (PHON-4) is located 5 km downstream of PHON-3 at a walkway bridge for foot traffic (Figure 2.2g-h). During the survey, water flow was visually rated as low, and water had a slight green colour. Weather consisted of sunny and warm conditions. The common water hyacinth (*Eichhornia crassipes*) was found floating on the water surface, while reeds and shrubs dominated the marginal vegetation. Hydraulic biotopes consisted of deep and shallow runs. The substrate consisted of large boulders, cobbles and sand-mud. Instream habitat was rated moderate to high in diversity. A dry floodplain pan within the Ndumo Game Reserve, indicated by point A on the map (Figure 2.1), was selected as a model site for the experimental microcosm study (see Chapter 5). The site is located approximately 150 m from the Phongolo River (Figure 2.2i-j). This pan represented a typical floodplain pool of the area that would receive water from the river during flooding events and knowledge on the aquatic biota of this pan has been documented in previous studies (Smit *et al.*, 2016; de Necker, 2016, 2019; de Necker *et al.*, 2022).

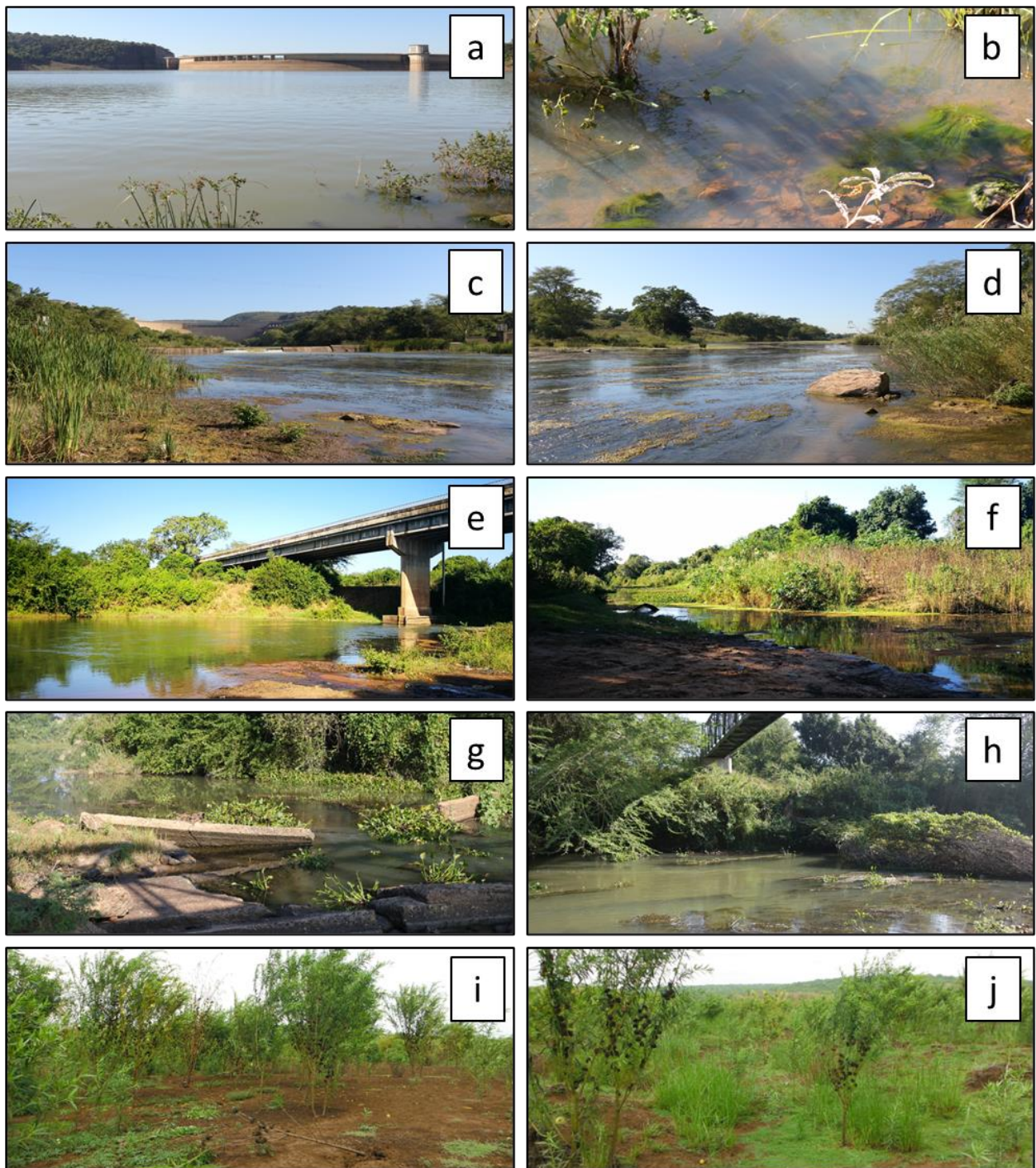


Figure 2.2: Photographs of the sites sampled on the Phongolo River from August to September 2017: (a) PHON-1 site on Pongolapoort Dam at Tiger Lodge and (b) a typical biotope at this site, (c-d) the PHON-2 site below the dam wall, (e-f) the PHON-3 located at a high-water bridge and, (g-h) the PHON-4 site and (i and j) the microcosm study location on a dry floodplain depression of the Phongolo River Floodplain within the Ndumo Game Reserve. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites. *Photo credit: Dr Lizaan de Necker.*

2.1.2 The Limpopo River system

The Limpopo River is one of the largest rivers (~1 750 km) in southern Africa (Mosase & Ahiablame, 2018). The river rises as the Crocodile River in the Witwatersrand and is joined by the Marico River forming the Limpopo River at the border between South Africa and Botswana (SARDC, 2003). The Limpopo River basin drains an area of ~415 000 km² in South Africa, Botswana, Zimbabwe and Mozambique (Mosase & Ahiablame, 2018). At the confluence of the Shashe River (the border between Botswana and Zimbabwe), the Limpopo River continues east to form the border between South Africa and Zimbabwe, before entering Mozambique at Pafuri (SARDC, 2003). It is joined by the Rio dos Elefantes River 209 km from the coast, where the river enters the Indian Ocean about 60 km downstream of the town Xai-Xai (SARDC, 2003). The Limpopo River system has 27 important watersheds, 12 of these watersheds are from South Africa (Mosase & Ahiablame, 2018). The average air temperature over the river system ranges between 0 to 36 °C from winter to summer (Mosase & Ahiablame, 2018). The Limpopo River system consists of arid climate conditions with an average yearly rainfall of 200 mm in the west to 1500 mm in the east, rainfall occurs between October and April during Austral summer (Mosase & Ahiablame, 2018). Rainfall in the river system differs between years, causing droughts during dry years and flood events during wet years (Mosase & Ahiablame, 2018). Increased agricultural activities, industrial development, and population growth place increased pressure on the Limpopo system's water resources (Van der Zaag *et al.*, 2010; Bawden *et al.*, 2014). Additionally, climate change adds uncertainty to the future availability of fresh water in the region (Mosase & Ahiablame, 2018).

The Crocodile River is the largest tributary of the Limpopo River (SARDC, 2003). The first site (CROC) sampled as part of the Limpopo River system took place on the Crocodile River (Figure 2.3a-b), next to the Rooibokkraal Road. The site is located just above the confluence with the Marico River (the start of the Limpopo River). During the survey, the weather was sunny to partly cloudy and warm. High water flow conditions were noted, and the water was olive green in colour and turbid. Water was cool and alkaline at the time of sampling. Marginal vegetation was limited to grass and reeds. Hydraulic biotopes consisted of eddies, deep and shallow runs, shallow riffles, and backwater. The substrate consisted of gravel beds, sand, and mud. Based on the diversity of water flow, turbulence, vegetation, depth and hydraulic biotopes present at the site, the instream habitat was rated moderate to low in diversity.

Flow conditions were also high at the LIMP-1 site on the Limpopo River (Figure 2.3c-d). The site is located 30 km downstream from the CROC site. Weather conditions were sunny and warm during the survey. The water was almost clear of colour with a slight green tint and was cool and found to be alkaline at the time of sampling. Due to recent high flow events, bank scouring was

observed. However, marginal vegetation was diverse: herbaceous plants, shrubs, grasses, and sedges were observed. Deep and shallow riffles, rapids, runs, large pools, backwater pools and eddies made up the hydraulic biotopes. Instream habitat was highly diverse, and substrates included bedrock, boulders, gravel, sand, mud and silt.

The Matlabas River rises in the Waterberg, flowing through the Marakele National Park and across the Lowveld, where it joins the Limpopo River (Marakele National Park, 2007). The MATL site on the Matlabas River had crystal clear water and moderate to low flow conditions (Figure 2.3e-f). During sampling, water was cool and neutral, while the weather consisted of sunny and warm conditions. The marginal vegetation was dominated by reeds, with few types of grass and sedges present. Hydraulic biotopes were limited, and the substrate was dominated by sand, gravel and mud. Based on the diversity of water flow, turbulence, vegetation, depth and hydraulic biotopes present at the site, the instream habitat was rated moderate to low in diversity.

On the Lephalala River, the fourth site, LEPH, is situated downstream of a weir about 15 km upstream from the confluence with the Limpopo River (Figure 2.3g-f). Sunny and warm weather conditions were noted at the time of sampling. High flow conditions were recorded while sampling this site. Water was cool, circum-neutral and slightly discoloured (light green to brown). Dominated by reeds, the marginal vegetation was also made up of very few types of grasses and sedges. The hydraulic biotopes consisted of shallow and intermediate riffles and -rapids and shallow pools, but the variety diminished further downstream. Downstream from the weir, instream habitat diversity was rated moderate to low, and substrates were made up of bedrock, boulders, and cobbles. However, sand-mud and sometimes gravel dominated further downstream.

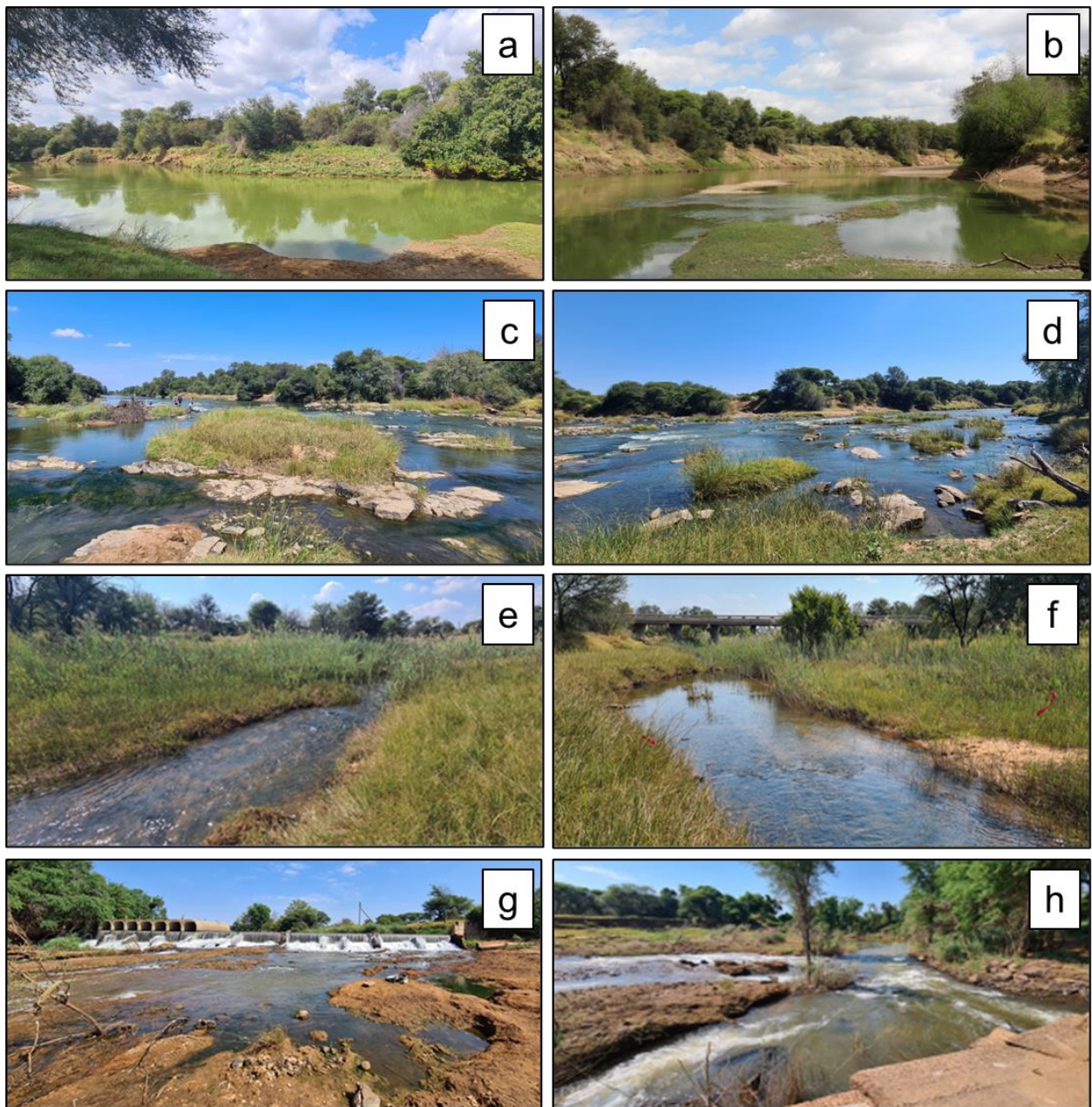


Figure 2.3: Photographs of sites on the Limpopo River system sampled from April to May 2021: (a-b) the CROC site sampled on the Crocodile River, (c-d) the LIMP-1 site on the Limpopo River, (e-f) the MATL site on the Matlabas River and (g-h) the LEPH site sampled on the Lephalala River. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites.

The second site on the main stem of the Limpopo River, LIMP-2, is located inside the Limpokwena Nature Reserve, about 300 km downstream from the LIMP-1 site (Figure 2.4a-b). The weather consisted of sunny and warm conditions during the survey. Water was cool and alkaline, while the flow was high with clear to red-brown water colouration. Due to bank scouring and high sand-mud deposition, marginal vegetation was limited, while the opposite riverbank had reeds, grasses and sedges. Hydraulic biotopes were made up of shallow to intermediate riffles and runs as well as medium pools. Deeper water was avoided for safety reasons due to the presence of Nile

crocodiles (*Crocodylus niloticus* Laurenti, 1768). Relatively high diversity of instream habitat occurred, and substrates included bedrock, boulders, cobbles, gravel and sand-mud.

Located on the Mogalakwena River, the MOGA site is the final site inside the Limpokwena Nature Reserve, two km upstream from the confluence with the Limpopo River (Figure 2.4c-d). Warm, sunny and windy weather conditions were noted during the survey. The water flow was restricted to a trickle close to the weir, and no visible flow was observed further downstream. Water was clear in colour and cool to warm, neutral to subsaline water conditions were noted. The site was devoid of marginal vegetation due to recent bank scouring during high flow events. Due to a lack of flow, hydraulic biotopes were limited to isolated pools. Due to the low water flow, turbulence, depth, hydraulic biotopes and no vegetation present at the site, instream habitat diversity was low. Substrates were limited to bedrock, cobbles, gravel and sand.

Inside the Mapungubwe National Park, the final site on the Limpopo River, LIMP-3, is located about 110 km downstream from the LIMP-2 site (Figure 2.4e-f). The weather consisted of sunny and warm conditions at the time of sampling. Water depth was primarily shallow, dominated by sand and fine gravel substrates. Moderate flow conditions were visible during the survey. Water was light brown, warm and alkaline at the time of sampling. Instream habitat diversity was low due to a lack of hydraulic biotopes, a low variety of depth-velocity classes and limited substrates.

On the Sand River, the SAND site falls in the Musina Nature Reserve, next to a high-water bridge on the R508 road (Figure 2.4g-h). The site is located approximately 19 km from the confluence with the Limpopo River, creating the border between South Africa and Zimbabwe. The weather was sunny and warm with slightly windy conditions. The site had shallow light brown water and poor flow conditions, while the water was warm and subsaline. Little marginal vegetation was present, mostly limited to reeds. The instream habitat was rated low in diversity due to a lack of hydraulic biotopes and limited depth-velocity classes. Cobbles, large boulders, and sand-mud-silt in slower flowing areas were present, while fine gravel and coarse sand were dominant substrates.

The LUVU site is on the Luvuvhu River, about one km from the Outpost Lodge inside the Kruger National Park (Figure 2.4i-j). During the survey, the weather consisted of hot and sunny conditions. The site was sampled during a very high flow event, with deep, fast-flowing sections. Water was clear to light brown, warm and alkaline. Grasses dominated marginal vegetation. Cobbles and large boulders primarily represented the substrate. Due to a wide variety of hydraulic biotopes and depth-velocity classes, instream habitat diversity was rated as high.

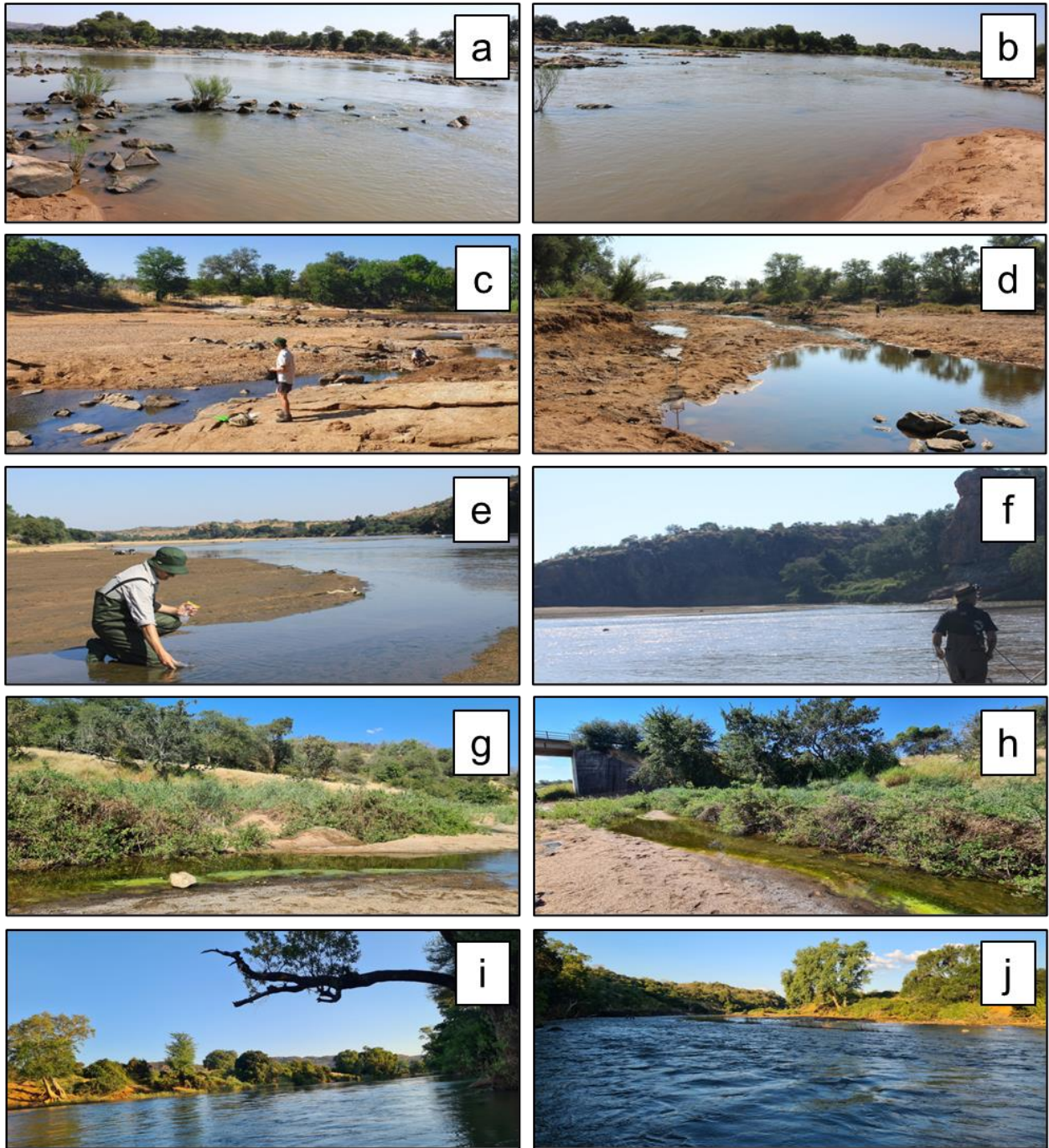


Figure 2.4: Photographs of sites on the Limpopo River system sampled from April to May 2021: (a-b) the LIMP-2 site on the Limpopo River inside the Limpokwena Nature Reserve, (c-d) the MOGA site sampled on the Mogalakwena River inside the Limpokwena Nature Reserve, (e-f) the LIMP-3 site on the Limpopo River inside the Mapungubwe National Park, (g-h) the SAND site sampled on the Sand River in the Musina Nature Reserve and (i-j) the LUVU site on the Luvuvhu River inside the Kruger National Park. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites.

The Olifants River rises at Trichardt (Mpumalanga Province, South Africa), flowing north-east through the Kruger National Park across the Lowveld, and is joined by the Letaba River inside the park (Nkhonjera, 2017). It continues through the Lebombo Mountains in the Olifants Gorge, becoming the Rio dos Elefantes River in Mozambique, flowing into the Massingir Dam and finally joining the Limpopo River further downstream (SARDC, 2003; Nhassengo *et al.*, 2021). The Olifants River is one of the most polluted and threatened river systems in South Africa due to the deteriorating water quality caused by mining, industrial and agricultural activities (de Villiers & Mkwelo, 2009; Van Vuuren, 2009; Kemp *et al.*, 2014; Gerber *et al.*, 2015; Addo-Bediako *et al.*, 2020). The first site on the Olifants River (OLIF-1) is inside the Kruger National Park, located 2.5 km downstream of the Mamba Gauging weir and 500 m downstream from the Olifants confluence with the Klaserie River. The first survey of the site in September 2020 indicated a high diversity of hydraulic biotopes (Figure 2.5a-b). The weather was cloudy and cool with slightly windy conditions. Water flow was low, algae were very dominant and marginal vegetation consisted of reeds and grasses. Sand and gravel were the dominant substrates, but bedrock, boulders and cobbles were also present. During the second survey in May 2021, the OLIF-1 site had a very high flow (Figure 2.6a-b). The water was slightly turbid and olive-green in colour. Sunny and warm weather conditions were noted during this survey. The water was cool and alkaline to subsaline. Marginal vegetation was dominated by grasses with some reeds present as well. Bedrock, cobbles, gravel, and sand were the dominant substrates. Instream habitat diversity was rated as high due to a wide variety of hydraulic biotopes and depth-velocity classes being present at the site.

The second site on the Olifants River, OLIF-2, is located at the Balule low-water bridge on the secondary S90 road between the Olifants and Balule rest camps inside the Kruger National Park (Figure 2.5b-c). The site is 70.6 km downstream from the first site and 5.7 km from the Olifants Rest Camp. There are 17 named intermittent streams flowing into the Olifants River between the OLIF-1 and OLIF-2 sites (Kloppers & Bornman, 2005). The larger tributaries entering the Olifants River between the first two sites include the Nharalumi, Timbavati, and Ngotso (Kloppers & Bornman, 2005). The Balule low water bridge forms an impoundment during flooding events, causing deposition of sand and gravel upstream of the bridge. Hydraulic biotopes indicated high diversity during both surveys, with increased turbidity and depth-velocity classes during the second survey. Due to the increase in water turbidity, flow and depth, sampling of deep areas were avoided for safety reasons. Loose gravel and cobbles on a deep bed of sand were the dominant substrates, with bedrock, mud, and silt present. Upon the second survey of the OLIF-2 site in May 2021 (Figure 2.6b-c), the weather consisted of hot and sunny conditions. The water was cool, alkaline and had a brownish colour, while the water flow was high. Reeds and grasses dominated the marginal vegetation. Substrate included bedrock, cobbles, gravel and sand. With

a wide variety of hydraulic biotopes and depth-velocity classes, the instream habitat diversity was rated high.

The last site on the Olifants, OLIF-3, is approximately 200 m upstream from the Olifants-Letaba confluence, 18 km downstream from the OLIF-2 site, and approximately nine km from the Olifants Rest Camp inside the Kruger National Park (Figure 2.5c-d). No large tributaries enter the Olifants River between the OLIF-2 and OLIF-3 sites, but intermittent streams include the Ninini, Shitsogwane, Wagdrifspruit and Bangwanene (Kloppers & Bornman, 2005). The Olifants-Letaba confluence joins at an angle that results in an upstream inundation of the Olifants River, leading to sand, gravel and cobble deposition. During the first field survey at the site in October 2020, most hydraulic biotopes were fast-flowing, with several freshly inundated areas due to rainfall events. Weather conditions consisted of cloudy and cold temperatures with light rain. As with the OLIF-2 site, the deeper biotopes were avoided due to safety reasons. Large boulders, cobbles, gravel and sand were the dominant substrates on a deep bed of sand with embedded cobbles and gravel. Mud and silt were limited to the inputs of intermittent streams. The site was not revisited during the second survey in 2021 due to safety reasons (Nile crocodiles, hippos (*Hippopotamus amphibius* Linnaeus, 1758) and the risk of drowning) following high flow events and difficulty of the terrain (very steep banks and many large boulders to climb over).

The Great Letaba River site (G-LETA) is located inside the Great Letaba Wildlife Reserve, about seven km from the confluence with the Letaba River (Figure 2.6e-f). The weather consisted of hot and sunny conditions during the survey. The site had moderate to low water flow conditions with a brownish colour and water was cool, alkaline to subsaline. Marginal vegetation was made up of grasses and reeds. Cobbles, bedrock and large boulders were limited, while sand and gravel were the dominant substrates. Hydraulic biotopes consisted of shallow riffles, runs and pools with moderate diversity in depth-velocity classes. Instream habitat was rated high in diversity. The second site on the Letaba River (LETA) is located approximately 10 km upstream of the Olifants-Letaba confluence, inside the Kruger National Park (Figure 2.6g-h). Cloudy and cool weather with low water flow conditions were noted during the survey. The water was cool, alkaline and almost clear with slight green-brown colour. Grasses dominated marginal vegetation. Bedrock, large boulders and sand-mud were well represented in the substrate, while sand and gravel were dominant. Instream habitat was high in diversity due to a variety of hydraulic biotopes and depth-velocity classes.

Situated on the Shingwedzi River, the SHIN site is approximately one km from the Mozambique border in the Kruger National Park (Figure 2.6i-j). The site is 140 km from the Shingwedzi-Elefantes River confluence and 240 km from the Elefantes-Limpopo confluence in Mozambique. The weather was overcast and cold during the survey. Moderate to low flow conditions were

observed. The water was cool, alkaline and had a brown colour. Marginal vegetation was limited to reeds and few types of grasses. The surface water was mostly restricted to isolated pools, deficient in hydraulic biotopes and depth-velocity classes. Thus, instream habitat diversity was rated as moderate.

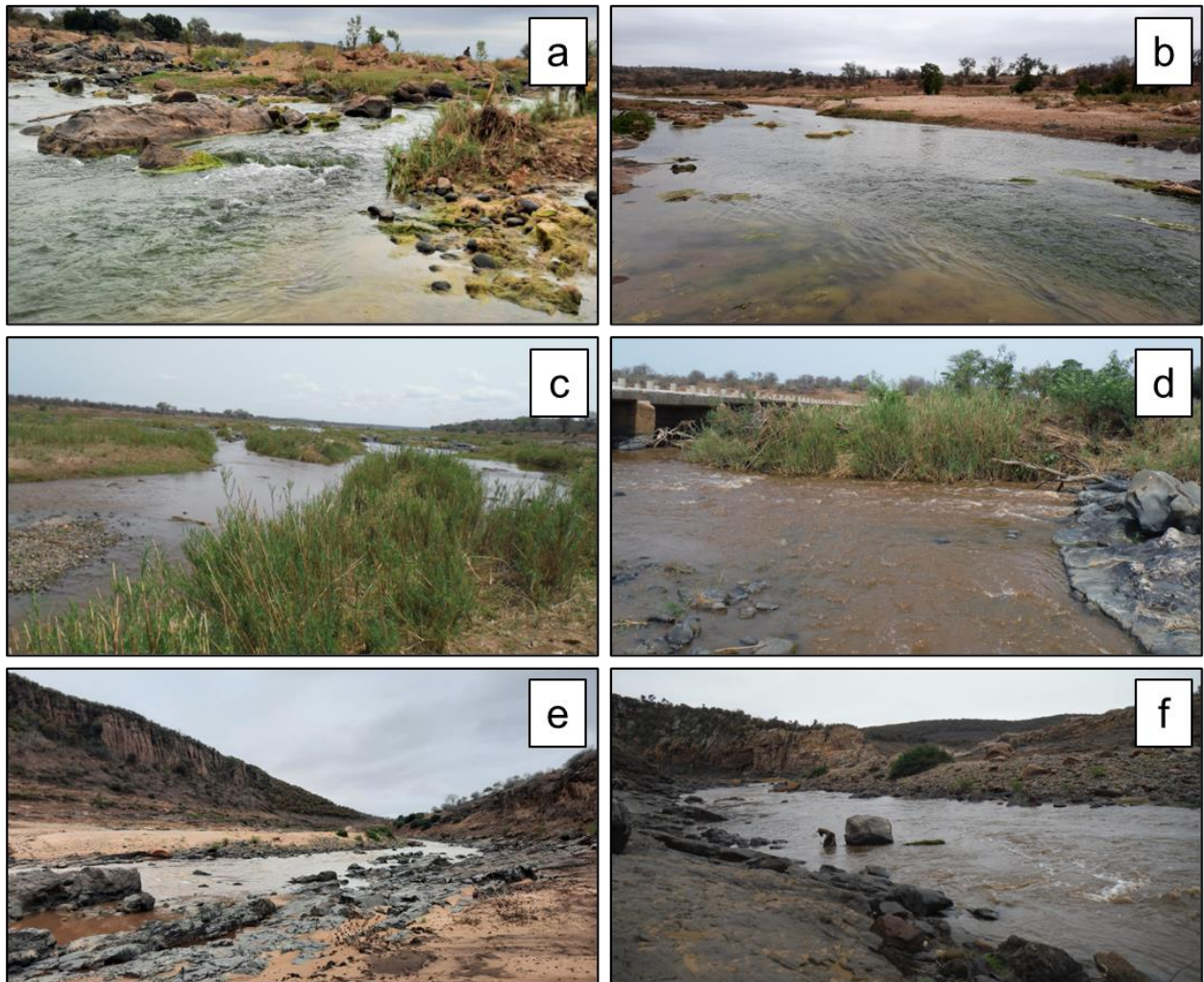


Figure 2.5: Photographs of sites on the Olifants River sampled from September to October 2020 in the Kruger National Park: (a-b) the OLIF-1 site downstream from Mamba weir, (c-d) the OLIF-2 site at Balule low-water bridge and (e-f) the OLIF-3 site upstream from the Olifants-Letaba confluence. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites.

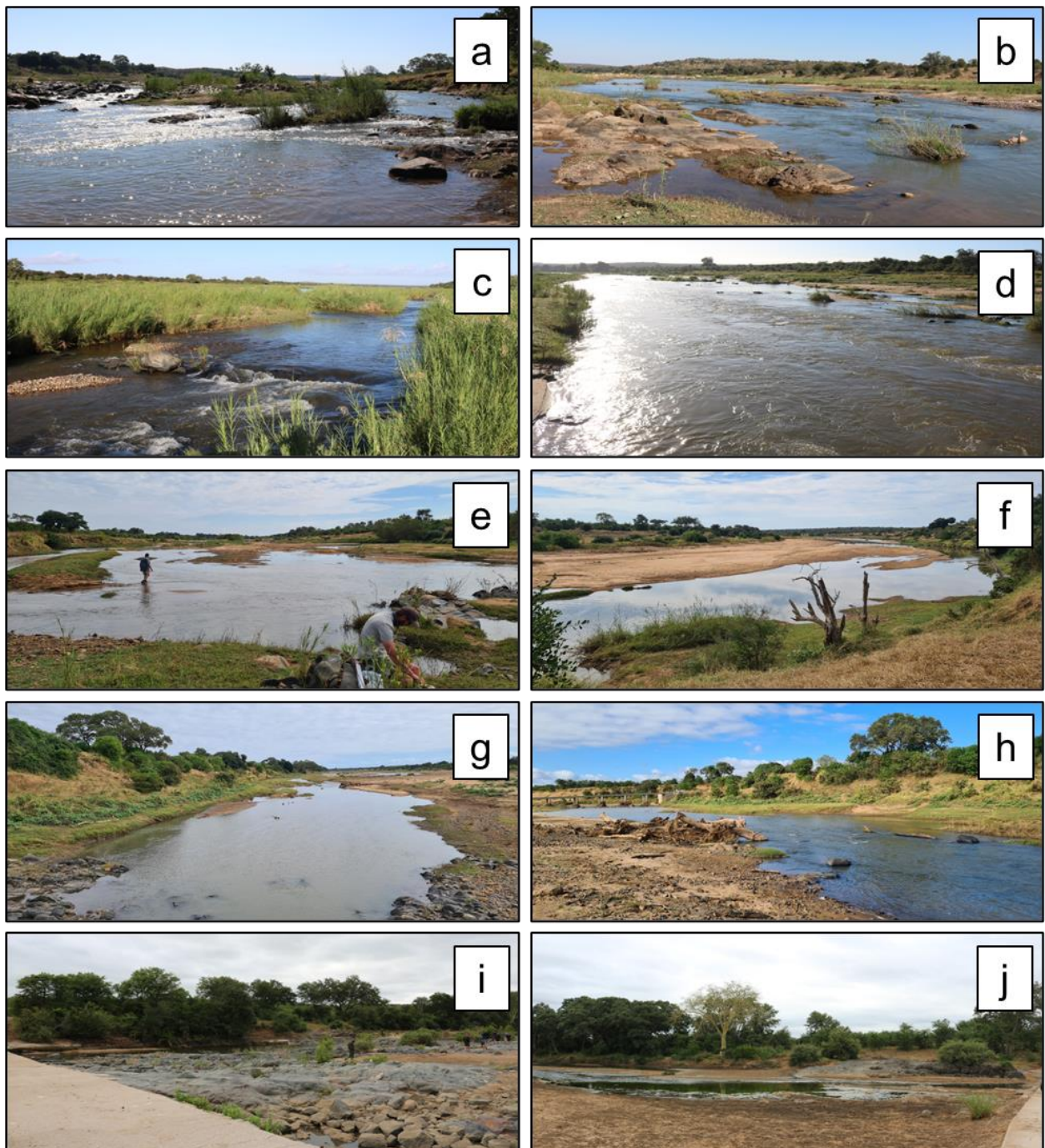


Figure 2.6: Photographs of sites on the Limpopo River system sampled from April to May 2021: (a-b) the OLIF-1 site downstream of Mamba weir, (c-d) the OLIF-2 site sampled on the Olifants River at Balule low-water bridge, (e-f) the G-LETA site on the Great Letaba River inside the Great Letaba Wildlife Reserve, (g-h) the LETA site sampled on the Letaba River and (i-j) the SHIN site on the Shingwedzi River. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites.

Chapter 3: Current distribution, densities and population structures of *Tarebia granifera* in the Limpopo River system and Phongolo River of South Africa

3.1 Introduction

Tarebia granifera is a highly resilient and opportunistic species as it can migrate upstream in fast-flowing water (Prentice, 1983; Snider & Gilliam, 2008; Appleton *et al.*, 2009), tolerate a wide salinity and temperature range (Miranda *et al.*, 2010) and have no natural predators (Miranda & Perissinotto, 2012; Miranda *et al.*, 2016). Furthermore, they can also flourish in highly disturbed environments (Jones *et al.*, 2017). These adaptations give them the ability to rapidly disperse and invade a wide variety of aquatic habitats in a relatively short time (Wolmarans & de Kock, 2006; Appleton *et al.*, 2009; Miranda *et al.*, 2010; Jones *et al.*, 2017). The rate and range of *T. granifera* invasion is a matter of great concern and needs to be investigated and closely monitored in the future (Wolmarans & de Kock, 2006; Appleton *et al.*, 2009; Weyl *et al.*, 2020).

The aquatic systems of the tropical and subtropical regions of KwaZulu-Natal, Limpopo, and Mpumalanga have been invaded by *T. granifera* (Appleton, 2003; Pointier *et al.*, 2003; Wolmarans & de Kock, 2006; Appleton *et al.*, 2009; Miranda *et al.*, 2010, 2011b; Dube *et al.*, 2017; Malherbe, 2018). The snail has been reported to cause declines in native snail species abundances due to their ability to reproduce quickly and reach high densities (Appleton, 2003; de Kock & Wolmarans, 2007; Appleton *et al.*, 2009; Miranda *et al.*, 2011a; Miranda & Perissinotto, 2014; Weyl *et al.*, 2020; Makherana *et al.*, 2022a). As *T. granifera* are usually the dominant invertebrate species in invaded aquatic communities (Pointier *et al.*, 1998; Canete *et al.*, 2004; Pillay & Perissinotto, 2008; Appleton *et al.*, 2009; Weyl *et al.*, 2020), they have a high probability of causing environmental harm due to their ability to reproduce rapidly (Parker *et al.*, 1999; Keller *et al.*, 2007; Rumi *et al.*, 2010; Nash & Hoffmann, 2012; Kesner & Kumschick, 2018).

To determine the interactions of invasive species with their environment and develop a suitable control strategy, it is important to understand their population dynamics (Burlakova *et al.*, 2010; Miranda *et al.*, 2011b). The density (number of individuals/m²) of a population describes the distribution and abundance of a species within a habitat (Ruehl & Trexler, 2011). Population size structures describes the size of individuals in a population and provides information on the growth rate, age at maturity, as well as reproductive status of individuals (Peters, 1983; Brown *et al.*, 2004; Ruehl & Trexler, 2011). Recent studies recommend reporting on both snail density and population size structures to understand the effects of a snail species on ecosystem function and community structures of freshwater ecosystems (White *et al.*, 2007; Ruehl & Trexler, 2011).

The density of a species can affect the biology of numerous other species in the same ecosystem (Conner *et al.*, 2008). For example, a decrease in the growth rate of native species can delay sexual maturity (reduced reproduction), causing an increase in mortality, and the displacement or total eradication of native snail species (Hillebrand *et al.*, 2002; de Kock & Wolmarans, 2008; Appleton *et al.*, 2009; Jones *et al.*, 2017; Miranda *et al.*, 2011a, 2011b; Miranda & Perissinotto, 2012, 2014). Many of the ecosystem changes occurring due to invasive species are irreversible due to the difficulty of removing such invaders from invaded habitats (Thresher & Kuris, 2004; Kesner & Kumschick, 2018).

Many studies have suggested that *T. granifera* could have negative effects on native aquatic snail communities (Hillebrand *et al.*, 2002; Appleton *et al.*, 2009; López-López *et al.*, 2009; Jones *et al.*, 2017; Pointier *et al.*, 2010; Miranda *et al.*, 2011a; Miranda & Perissinotto, 2014) and research on the impacts of this invader on native snail community structures and biodiversity are urgently needed (Appleton *et al.*, 2009; Weyl *et al.*, 2020). Studies on the population dynamics of an invasive species can provide information on how these species interact with newly invaded environments (Miranda *et al.*, 2011b). Over time, fluctuations in the population densities and size structures of *T. granifera* can reveal how these invasive snail populations survive and recover during unfavourable conditions such as drought, flooding or increased water salinity (Miranda *et al.*, 2011b). Previous studies from South Africa reported differences in *T. granifera* density due to differences water quality variables (Jones *et al.*, 2017; Makherana *et al.*, 2022a).

This chapter aimed to investigate the current distribution, densities, and population size structures of *T. granifera* at selected sites on the Limpopo River system and Phongolo River. Key interests included: 1) using snail body size to determine the age of maturity and reproductive status of snail populations at the selected sites sampled, 2) whether there is a relationship between *T. granifera* density and selected water quality parameters, 3) whether native snail diversity and distribution might be influenced by high *T. granifera* density. The hypotheses for this chapter were: 1) *T. granifera* will occur at densities greater than within their native range and population structures will reflect an established population with an even spread of individuals throughout the size classes, 2) *T. granifera* density are related to water quality parameters such as pH, conductivity and temperature, and 3) high densities of invasive *T. granifera* is expected to correlate with low native mollusc densities and diversity, suggesting that the invader can displace native mollusc communities.

3.2 Materials and methods

3.2.1 Water sample collection

Water quality data for the first two sites on the Phongolo River (Appendix A1) were obtained from the Department of Water and Sanitation (DWS) National Chemical Monitoring Programme and de Necker (2019). The first two sites' data (PHON-1 and PHON-2) were measured from 2016 to 2017 at the DWS gauging stations. Data for PHON-1 sites was measured at the W4H006 gauging station located upstream of Pongolapoort Dam, data for the PHON-2 site was measured at the W4H013 gauging station located below the dam wall. The water quality data assessed at these stations included electrical conductivity (EC; $\mu\text{S}/\text{cm}$), pH, chloride (Cl^-), sulphates (SO_4^{2-}), nitrate (NO_3^-), ammonium (NH_4^+) and orthophosphates (PO_4^{3-}).

The last two sites on the Phongolo River (PHON-3 and PHON-4), all sites sampled on the Olifants River (part of the Limpopo River system) in the Kruger National Park from September to October 2020 and April to May 2021 (Appendix A2) as well as the sites on the Limpopo River system sampled between April and May 2021 (Appendix A3) were collected as described below. Physiochemical water quality variables: pH, temperature ($^{\circ}\text{C}$), EC ($\mu\text{S}/\text{cm}$), total dissolved solids (TDS) (mg/L), dissolved oxygen (mg/L) and oxygen concentration (%) was measured *in situ* at each site using Extech EC500 pH/electrical conductivity and Extech DO600 dissolved oxygen water quality meters. Water samples (250ml) from each site were also collected in triplicate for chemical nutrient analyses. Samples were preserved by freezing (-4°C) them on-site in a mobile freezer until analysis could be conducted in the laboratory at the North-West University (NWU). In the laboratory, water samples were left to defrost at room temperature and analysed for the following chemical nutrient variables: Turbidity (FAU), SO_4^{2-} , NH_4^+ , NO_3^- , NO_2^- , PO_4^{3-} , Cl^- , and chemical oxygen demand (COD) (mg/L) using the appropriate test kits and a Merck Spectroquant Pharo 300 Spectrophotometer (Merck KGaA, 2014).

3.2.2 Snail sampling and identification

Each of the 19 selected sites was sampled for all available aquatic benthic molluscs (including snails and bivalves/clams), using a snail sampler. The sampler is made of an aluminium flat frame with a sieve (30 x 30 cm, 2 mm mesh size) and handle. The snail sampler was used to scoop up sediment and sieve through the benthic zone of the river (Figure 3.1a), three replicates made up of ten scoops per replicate (30 sampler scoops) were collected at each site. Sampling occurred randomly within the site areas, avoiding deep waters due to the presence of crocodiles at some sites. Samples were preserved in plastic containers with 70% ethanol and transported back to the laboratory (NWU) where the molluscs were identified to species level according to Appleton

(2002). Species were divided into “live” or “dead” individuals where individuals were considered dead when their shells did not contain any tissue and the shells were usually degraded and started to lose colour (Figure 3.1b). All individuals that were considered “live” at the time of sampling were then counted (Figure 3.1c) to subsequently calculate the density of each species per site (number of individuals sampled/m²).

The population size structures were then determined for the invasive thiarid (*T. granifera*) and for the native thiarid (*Melanoides tuberculata*). Due to the extremely high densities and often quite small sizes of *T. granifera*, not all measurements could be conducted by hand and the following method was thus used for accurate size determination: The snail samples were dried in an oven (50 °C) for 48 hours. A King Test (VB 200 300) sieve shaker with Clear Edge Test sieves was then used to sort the snails into size classes, the sieves were stacked on each other from largest mesh size to smallest, with a collection pan at the bottom (Figure 3.1d). A sample of 1 070 snails was placed in the top sieve and shaken for 10 min. The snails passed through the sieves according to the mesh size of the sieves (4000, 2000, 1000, 500 µm), dividing them into size classes according to the width of the snail shells. The length of snails from each size class was then measured by hand (Figure 3.1e) with a digital calliper (Kroeplin B2R20S) with an accuracy of 0.1 mm and counted, from these findings a size class according to snail length was developed for each of the different sieve sizes (Table 3.1).

All *T. granifera* from all sampled sites with a width > 4 mm (length > 10.39) were measured in length with a digital calliper, from the anterior end to the posterior end of the shell (Samsi *et al.*, 2020). All snails with a width ≤ 4 mm (length < 10.39) were divided into size classes according to length (Table 3.1). *Melanoides tuberculata* size classes were determined in the same manner as for *T. granifera*. For this study, size classes were used to describe age groups based on the size of sexual maturity in the populations obtained from published literature (Figure 3.1f). *Tarebia granifera* were divided into mature adults (> 10.39 mm) that are considered able to reproduce, adults (5.28 - 10.39 mm) that are considered becoming sexually mature and starting to reproduce, juveniles (1.92 - 5.27 mm) that cannot reproduce, and new-born snails (< 1.9 mm) that were born recently (Abbott, 1952; Chaniotis *et al.*, 1980a; Prentice, 1983; Appleton *et al.*, 2009; Miranda *et al.*, 2011b). *Melanoides tuberculata* size classes consisted of older adults (> 12.64 mm) that were considered to be able to reproduce, mature adults (6.25 – 12.64 mm) that were also considered to be able to reproduce, adults (2.33 – 6.24 mm) that were becoming sexually mature, and juvenile snails (< 2.33 mm) that were unable to reproduce (Dudgeon, 1986). The average length, length range and standard deviation of mature adult *T. granifera* (> 10.39 mm) and older adult *M. tuberculata* (> 10.39 mm) were calculated.



Figure 3.1: Methods used to collect and process aquatic snails, a) collecting snails with an aluminium snail sampler, b) comparison of live snails (left) and dead snails (right), c) snails being identified to species level and counted, d) the King Test (VB 200 300) sieve shaker with Clear Edge Test sieves used to sort snails into size classes, e) measuring snail length in mm with a digital calliper, and f) size comparison of a mature adult and a juvenile *Tarebia granifera*.

Table 3.1: Method used to divide *Tarebia granifera* with a width ≤ 4 mm (length ≤ 10.39) into size classes according to length.

Shaker sieve size (μm)	4000	2000	1000	500
Snail width range (mm)	> 4	4-2	2-0.5	< 0.5
Minimum snail length (mm)	10.04	5.28	1.92	< 1.9
Maximum snail length (mm)	> 10.04	10.39	5.27	1.9
Snail abundance out of 1 070	62	583	391	34
Snail length size class (mm)	> 10.39	10.39-5.28	5.27-1.92	< 1.92
Snail age group	Mature adults	Adults	Juveniles	New-borns

Tarebia granifera birth rate was also calculated from the three sites sampled on the Olifants River between September and October 2020. Adult *T. granifera* snails gave birth while they were housed to investigate the possibility of parasitic infection (Chapter 5). The new-born snails were collected, and the birth rate was calculated: Birth rate = $N_b/N_a/N_d$. Where N_b is the number of juvenile snails born, N_a is the number of adult snails housed and N_d is the number of days the snails were housed. All samples during the present study were collected and processed with the

necessary permits from the Limpopo Department of Economic Development, Environment and Tourism (Permit No. ZA/LP/107276), Ezemvelo KZN Wildlife (Permit No. OP 941/2016, OP 1075/2017 and OP 864/2021) and ethical clearance from the North-West University AnimCare Animal Research Ethics Committee (Ethics No. NWU-01539-20-A9).

3.2.3 Statistical analysis

GraphPad Prism 7 was used to create graphical representations of snail density (m^2) and snail population size contributions as well as tests for significant differences. Differences in snail density for each of the species in each of the sampled systems were tested for significance using a Kruskal-Wallis test for non-parametric data and Dunn's post hoc test to determine specific significant ($p < 0.05$) pairwise differences between the densities of *T. granifera* versus the other snail species sampled from each system (Stoline, 1981; de Necker, 2019) e.g., "*Tarebia granifera* vs. *Corbicula fluminalis*", "*Tarebia granifera* vs. *Melanoides tuberculata*", "*Tarebia granifera* vs. *Physella acuta*". Pearson's correlation tests were completed to determine whether *T. granifera* density were significantly correlated to water quality variables.

3.3 Results

From the 19 selected sites sampled, aquatic molluscs were found to be present at 14 sites. *Tarebia granifera* were the only species that were present at all 14 of these sites. No molluscs were found at the CROC, MATL, LEPH, SAND, and LIMP-3, sites during the study. A total of four mollusc species were collected during the study, two invasives: *T. granifera* and *Physella acuta* and two natives: *M. tuberculata* and *Corbicula fluminalis*. In total 29 378 aquatic molluscs were sampled, counted and measured, *T. granifera* were the dominant species making up 26 178 individuals, followed by *C. fluminalis* (1 596 individuals), *M. tuberculata* (1 456 individuals) and *P. acuta* (148 individuals).

3.3.1 *Tarebia granifera* density

Tarebia granifera was present at all four sites sampled on the Phongolo River (Figure 3.2), with an average density of 699 individuals/ m^2 between sites. The PHON-2 and PHON-3 sites had the highest densities of *T. granifera* (1 648 and 610 individuals/ m^2 respectively) while the PHON-1 and PHON-4 sites had the lowest densities (319 and 217 individuals/ m^2 respectively). The highest snail densities recorded during the entire study (all 14 sites) were of *T. granifera* from the OLIF-1 and OLIF-2 sites during the 2020 survey on the Olifants River with 3 290 and 3 183 individuals/ m^2 respectively. The same two sites (OLIF-1 and OLIF-2) sampled in 2021 had much lower densities of *T. granifera* (48 and 564 individuals/ m^2 respectively). The OLIF-3 site (surveyed in 2020) had a density of 519 individuals/ m^2 . The OLIF-3 site was not revisited during the second survey in

2021 due to safety reasons following high flow events and the difficulty of the terrain (very steep banks and many large boulders to climb over). The three sites sampled on the Limpopo River had low densities of the invasive *T. granifera*. The highest density of the snail was recorded at the second site on the Limpopo River (LIMP-2) with 15 individuals/m² followed by the first site (LIMP-1) with three individuals/m², while the LIMP-3 site did not have any snails present. The sites on the Mogalakwena (MOGA) and Great Letaba (G-LETA) rivers had higher densities of *T. granifera* (136 and 214 individuals/m² respectively). While the sites on the Shingwedzi River (SHIN; 67 individuals/m²), Letaba River (LETA; 56 individuals/m²) and the Luvuvhu River (LUVU; 42 individuals/m²) had lower densities of the invasive snail.

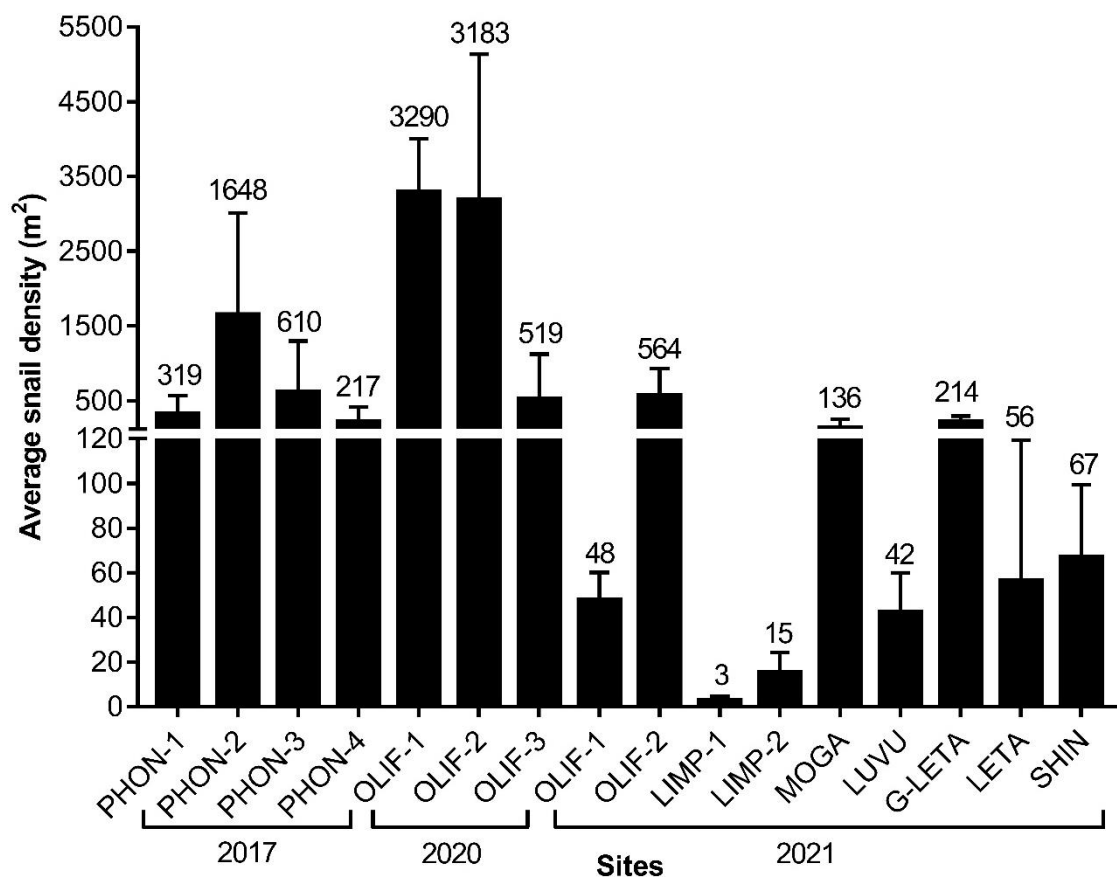


Figure 3.2: Average *Tarebia granifera* population density ($\pm$ SD) sampled from the selected sites on the Phongolo River and Limpopo River system from 2017 to 2021.

Results from Pearson's correlation tests (Table 3.2) indicated no significant correlations between *T. granifera* density and any of the water quality variables ($p > 0.05$; $R^2 < 0.06$).

Table 3.2: Pearson correlations between *Tarebia granifera* density and water quality variables of all sites sampled with *T. granifera* on the Phongolo River and Limpopo River system.

	Temperature (°C)	Conductivity (µS/cm)	Water quality variables					
			pH	Cl ⁻ (mg/L)	SO ₄ (mg/L)	NO ₃ (mg/L)	NH ₄ (mg/L)	PO ₄ (mg/L)
<i>T. granifera</i> density (m²)	R ² = 0.034 p= 0.50	R ² = 0.021 p= 0.59	R ² = 0.0001 p= 0.95	R ² = 0.026 p= 0.55	R ² = 0.056 p= 0.38	R ² = 0.046 p= 0.42	R ² = 0.0054 p= 0.79	R ² = 0.009 p= 0.73

3.3.2 *Tarebia granifera* population size structures

The average birth rate of *T. granifera* was 1.89 new-born snails per day. The percentage contribution of *T. granifera* population size structures are illustrated in Figure 3.3 and described below. The *T. granifera* population from the Phongolo River (PHON) mostly consisted of adults (42.51%) and mature adults (41.30%) from the 5.28 - 10.39 and > 10.39 mm size classes respectively. While 15.38 and 0.81% of the population consisted of juveniles (1.92 - 5.27 mm) and new-borns (< 1.9 mm) respectively. During the 2020 survey to the Olifants River, the OLIF-1 population consisted of mostly adults (79%), followed by juveniles (14%) while few mature adults (5.89%) and new-borns (0.66%) were present. The OLIF-2 population also had few mature adults (5.82%) while juveniles and new-born *T. granifera* were more abundant (48 and 27.82%, respectively). The contribution of new-borns was the highest recorded across all populations from all sites sampled. Adults (37.59%) dominated the population of the OLIF-3 population, followed by juveniles (31%), new-born *T. granifera* (18.62%) and mature adults (11.84%). The *T. granifera* population structure at OLIF-1 (2021) did not differ much temporally and the population consisted of mostly adults (55.81%) and juveniles (30%); juveniles increased by 16% from 2020 to 2021. While mature adults again formed the smallest part of the population (13.95%) even though it increased by 8.06% from 2020 to 2021, no new-born *T. granifera* were sampled during the 2021 survey. The population of the OLIF-2 site (2021) indicated an increase in juveniles from 48 to 61%, adults from 17.76 to 21.72%, and mature adults from 5.82 to 16.80% while the relative contribution of new-borns declined from 27.82 to 0.33%. The juvenile population sampled from the OLIF-2 site during the 2021 survey, were the highest recorded contribution of juveniles from all populations across all sites sampled.

For both the LIMP-1 and LIMP-2 sites on the Limpopo River, no new-born *T. granifera* were sampled and adult snails formed the greatest part of the populations, 57.1 and 82.93%, respectively. The adult population of the LIMP-2 site were the highest relative contribution for all population ages across all sites. Juvenile and mature adults contributed to 28 and 14.29% of the LIMP-1 snail population, while they contributed to 7 and 9.76% of the LIMP-2 population,

respectively. The *T. granifera* population of the Mogalakwena River (MOGA), had the greatest proportion of mature adults (64.95%) of all the populations from the selected sites. Adults and new-borns had similar contributions (16.58 and 14.95%, respectively) while juveniles formed the smallest proportion of the population (3%). This was also lowest contribution of juveniles and adults recorded for all *T. granifera* populations. The population from the Luvuvhu River site (LUVU), had the highest contributions of mature adults (55.26%) followed by adults (31.58%), juveniles (7%) and new-borns (5.26%). On the Great Letaba River, G-LETA populations mainly consisted of juveniles (56%) followed by adults (27.51%), mature adults (15.05%) and new-borns (1.38%). The lowest contribution of mature adults (5.26%) from all *T. granifera* populations was recorded for the LETA population on the Letaba River. Adults (75.66%) contributed the most to the LETA population followed by juveniles (18%) and new-borns (0.66%). The last population sampled, on the Shingwedzi River site (SHIN), followed the same trend as the LETA population. Adults (61.33%) contributed the most followed by juveniles (26%), mature adults (7.73%) and new-borns (4.42%).

Shell length (average $\pm$ SD) and length range (mm) of mature adults *T. granifera* sampled from the selected sites are summarized in Table 3.3. From all sampled sites, mature adult *T. granifera* shell length ranged from 10.40 to 25.21 mm and had an overall average length of 13.39 mm ($\pm$ 1.12).

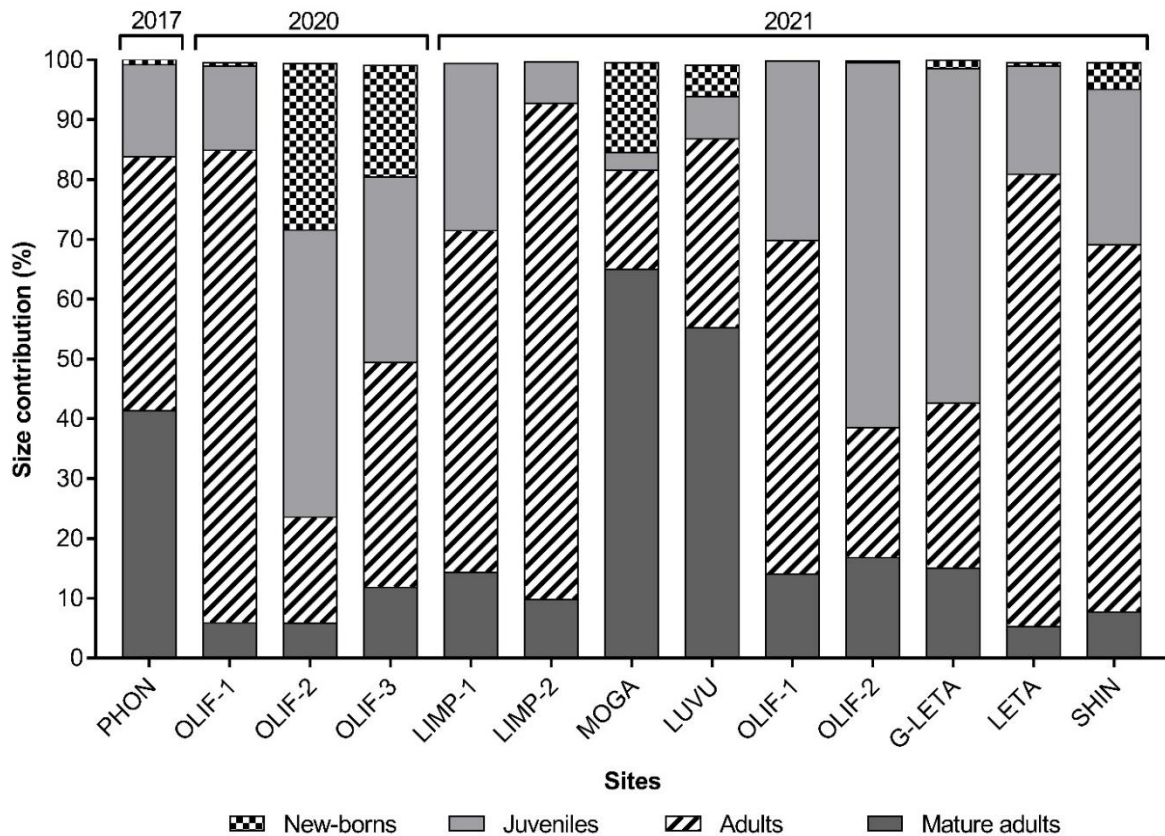


Figure 3.3: Percentage size contributions of *Tarebia granifera* populations from all selected sites sampled from 2017 to 2021. Snails were divided into mature adults (> 10.39 mm), adults (5.28 - 10.39 mm), juveniles (1.92 - 5.27 mm), and new-borns (< 1.9 mm).

Table 3.3: Average density (m²), shell length (average $\pm$ SD) and length range (mm) of mature adult *Tarebia granifera* (> 10.38 mm) sampled at the selected sites. Average densities were rounded up to the closest whole number. * Indicates Olifants sites sampled in 2021.

Site	Average density (m ²)	Length in mm (Average $\pm$ SD)	Length range (mm)
<i>Tarebia granifera</i> > 10.39 mm			
PHON	113	13.12 $\pm$ 2.63	10.43 - 25.21
OLIF-1	194	13.09 $\pm$ 2.31	10.40 - 20.63
OLIF-2	185	15.16 $\pm$ 1.85	10.41 - 20.34
OLIF-3	61	13.44 $\pm$ 2.23	10.42 - 19.87
LIMP-1	2	15.56 $\pm$ 3.15	10.45 - 15.56
LIMP-2	1	11.23 $\pm$ 0.68	10.57 - 12.27
MOGA	88	13.12 $\pm$ 1.88	10.45 - 20.54
LUVU	23	13.22 $\pm$ 1.54	10.80 - 17.04
OLIF-1*	7	12.46 $\pm$ 1.62	10.48 - 16.87
OLIF-2*	95	14.38 $\pm$ 1.78	10.45 - 20.52
G-LETA	32	12.18 $\pm$ 1.65	10.40 - 18.57
LETA	3	13.62 $\pm$ 1.63	11.26 - 15.88
SHIN	5	13.55 $\pm$ 3.50	10.40 - 20.54

3.3.3 Other mollusc diversity, density and community size structures

The percentage contribution of all mollusc species sampled at each site are illustrated in Figure 3.4. and described below. A total of four mollusc species (*T. granifera*, *C. fluminalis*, *M. tuberculata* and *P. acuta*) were sampled from the Phongolo River with an average density of 731 individuals/m² sampled from the four sites. *Tarebia granifera* were the dominant species sampled from the four sites on the Phongolo River, the snail had the highest percentage abundances at the PHON-3 and PHON-4 sites, contributing up to 99.46 and 99.39% of the total mollusc community sampled at the sites respectively. While the PHON-1 and PHON-2 sites had the lowest *T. granifera* abundances (92.30 and 94.49% respectively). *Corbicula fluminalis* made up 1.92, 0.32 and 0.61% of the total mollusc community at the PHON-1, PHON-3, and PHON-4 sites respectively. *Physella acuta* were present at the PHON-2 and PHON-3 sites contributing 5.51 and 0.22% respectively. While *Melanoides tuberculata* were collected only at the PHON-1 site contributing to 5.78% of the mollusc community at the site.

From the three sites sampled on the Olifants River (OLIF-1, OLIF-2, and OLIF-3) during the 2020 survey, a total of three species (*C. fluminalis*, *P. acuta* and *T. granifera*) were collected with an average density of 2480 individuals/m² sampled from the three sites. While only two mollusc species (*C. fluminalis* and *T. granifera*) were collected from the two sites on the Olifants River sampled during the 2021 survey (OLIF-1 and OLIF-2) with an average density of 338 individuals/m² sampled from the two sites. *Tarebia granifera* were the dominant snail species during both the 2020 and 2021 surveys. During the 2020 survey, the OLIF-1 and OLIF-2 sites had the highest percentage abundances of *T. granifera* (94.34 and 98.07% respectively) while the OLIF-3 site had the lowest abundance (73.40%). *Corbicula fluminalis* contributed to 5.54, 1.86 and 25.92% and *P. acuta* to 0.12, 0.07, and 0.68% of the total mollusc communities, respectively. During the 2021 survey, *T. granifera* contributed to 77.71% and *C. fluminalis* to 22.29% of the mollusc community at the OLIF-1 site, while *T. granifera* contributed to more (91.81%) and *C. fluminalis* (8.19%) less at the OLIF-2 site. No *P. acuta* were found at the two sites on the Olifants River during the 2021 survey.

A total of three species (*C. fluminalis*, *M. tuberculata*, and *T. granifera*) were collected on the Limpopo River with an average density of 283 individuals/m² sampled from the two sites (LIMP-1 and LIMP-2). No molluscs were present at the third site (LIMP-3) on the Limpopo River. *Melanoides tuberculata* were the dominant species at the LIMP-1 site, contributing 98.62% of the total mollusc community sampled at the site, followed by *C. fluminalis* and *T. granifera* (0.90 and 0.48% respectively). At the second site (LIMP-2) *T. granifera* and *C. fluminalis* had similar contributions to the community (51.25 and 48.75%, respectively). The Letaba River site (LETA) had three species of molluscs (*C. fluminalis*, *P. acuta*, and *T. granifera*) with an average density

of 118 individuals/m². The dominant species was *C. fluminalis*, contributing 51.57% of the total community sampled at the site, followed by *T. granifera* (47.80%) and *P. acuta* (0.63%). The entire molluscan community (100%) at the LUVU site on the Luvuvhu River consisted of *T. granifera* (with a density of 42 individuals/m²) and this was the only site with a single species and the lowest mollusc diversity (excluding the five sites where no molluscs were present). From the remaining three sites on the Limpopo River system, only *C. fluminalis* and *T. granifera* were present during the surveys at each site. The average mollusc densities at these sites were: G-LETA (215 individuals/m²), MOGA (137 individuals/m²) and SHIN (69 individuals/m²). *Tarebia granifera* was the dominant species at all three sites, contributing to 99.48 (G-LETA), 99.27 (MOGA), and 96.80% (SHIN) of the mollusc communities sampled at each site, while *C. fluminalis* contributed only to 0.52, 0.73, and 3.20%, respectively.

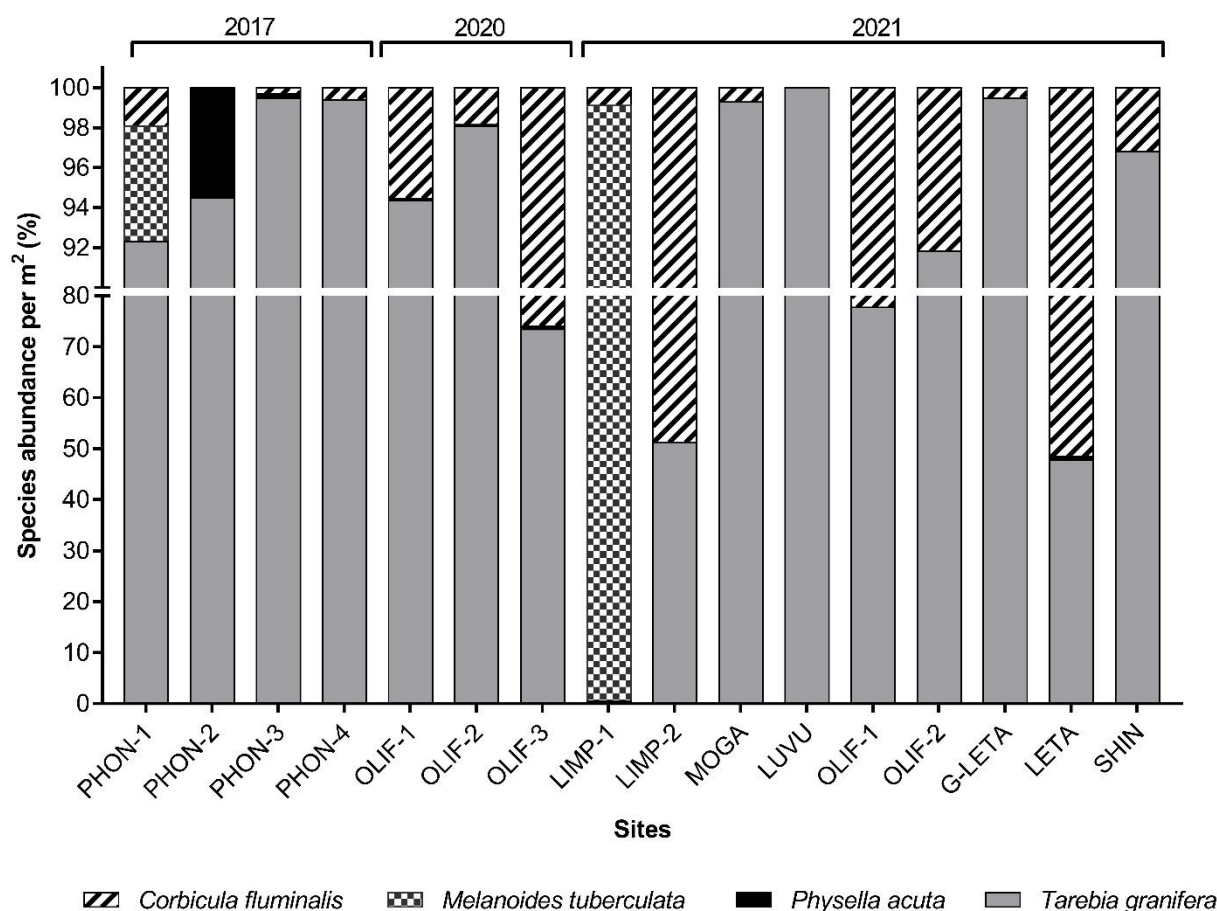


Figure 3.4: Percentage contribution of mollusc communities per site of all species sampled from the selected sites between 2017 to 2021.

On the Phongolo River where the invasive *T. granifera* dominated across all four sites (Figure 3.5), the invasive *P. acuta* were the species with the second highest average density at the four sites (24 individuals/ m²). The highest density for *P. acuta* was recorded at the PHON-2 site (96 individuals/ m²), while only one individual/m² was recorded at the PHON-3 site. The native *C.*

fluminalis had the following densities: PHON-1 (seven individuals/m²), PHON-3 (two individuals/m²), and PHON-4 (one individual/m²), while the native *M. tuberculata* had a density of 20 individuals/m² at the PHON-1 site. “Dead” individuals (empty shells) as described in section 3.2.2, of Planorbidae (Rafinesque, 1815) species (4 dead individuals/m²) were also present in the samples of the PHON-1 site (Appendix A4). This is an indication that these snails must have existed within the Phongolo study area in the past.

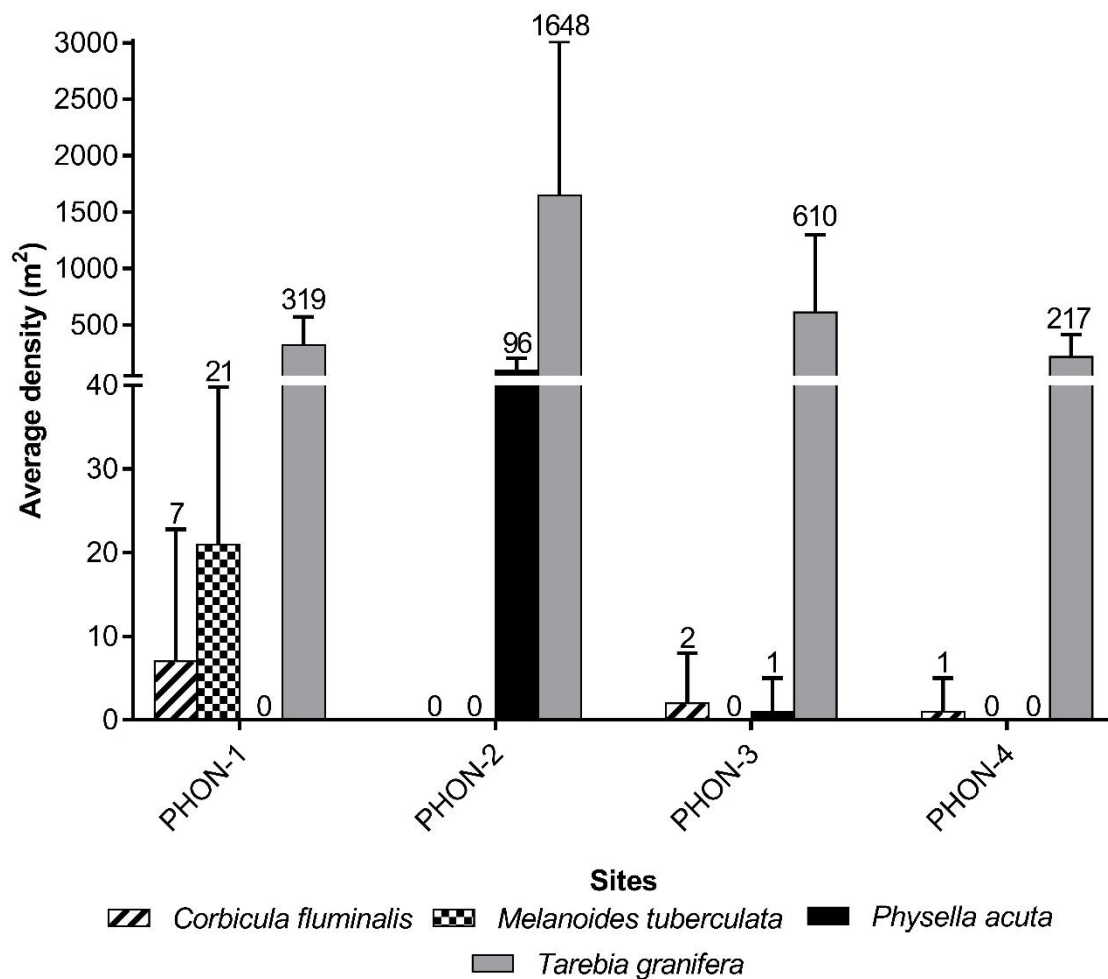


Figure 3.5: Average density ($\pm$ SD) of mollusc species sampled from the four sites on the Phongolo River between August and September 2017.

With the exception of two sites (LIMP-1 and LETA), *T. granifera* was the dominant molluscan species at all sites sampled on the Limpopo River system. During the two surveys to the Olifants River (2020 and 2021), the native *C. fluminalis* were sampled at all sites during both surveys (Figure 3.6). The highest densities of *C. fluminalis* were sampled at the OLIF-1 (193 individuals/m²) and OLIF-3 (183 individuals/m²) sites during 2020, while the lowest densities were recorded during 2021 at the OLIF-1 (14 individuals/m²) and OLIF-2 (50 individuals/m²) sites. The invasive *P. acuta* was only sampled from the three sites on the Olifants River during the 2020

survey, with the highest density recorded from the OLIF-3 (five individuals/m²) and OLIF-1 (four individuals/m²) sites, while the OLIF-2 site had a density of two individuals/m². The LIMP-1 site on the Limpopo River (Figure 3.7) was dominated by the native *M. tuberculata* (528 individuals/m²) followed by *C. fluminalis* (five individuals/m²) while the invasive *T. granifera* were present at a low density. At the second site on the Limpopo River (LIMP-2), *C. fluminalis* (14 individuals/m²) had similar low densities than the invasive *T. granifera* (also see section 3.3.1). The LIMP-2 site was also the site with the lowest average mollusc density (29 individuals/m²) of all sites. The LETA site on the Letaba River, *C. fluminalis* (61 and individuals/m²) and *T. granifera* also had similar densities, while *P. acuta* density was low (one individual/m²). The LUVU site on the Luvuvhu River had the second-lowest average mollusc density of which all were *T. granifera*. On the Shingwedzi River, the SHIN site was dominated by *T. granifera* while a very low density of *C. fluminalis* was sampled (two individuals/m²). The sites on the Mogalakwena (MOGA) and Great Letaba (G-LETA) rivers also had very low *C. fluminalis* densities (one individual/m² at both sites) while *T. granifera* were dominant. “Dead” (as described in section 3.2.2) *Bulinus tropicus* (Krauss, 1848) were present in the samples collected at the LIMP-1 (two individuals/m²) and SHIN (one individual/m²) sites and “dead” *Bulinus forskalii* (Ehrenberg, 1831) were also collected from the MOGA site (one individual/m²) samples (Appendix A6). This is an indication that these snail species must have been present in the Limpopo River system study area in the past.

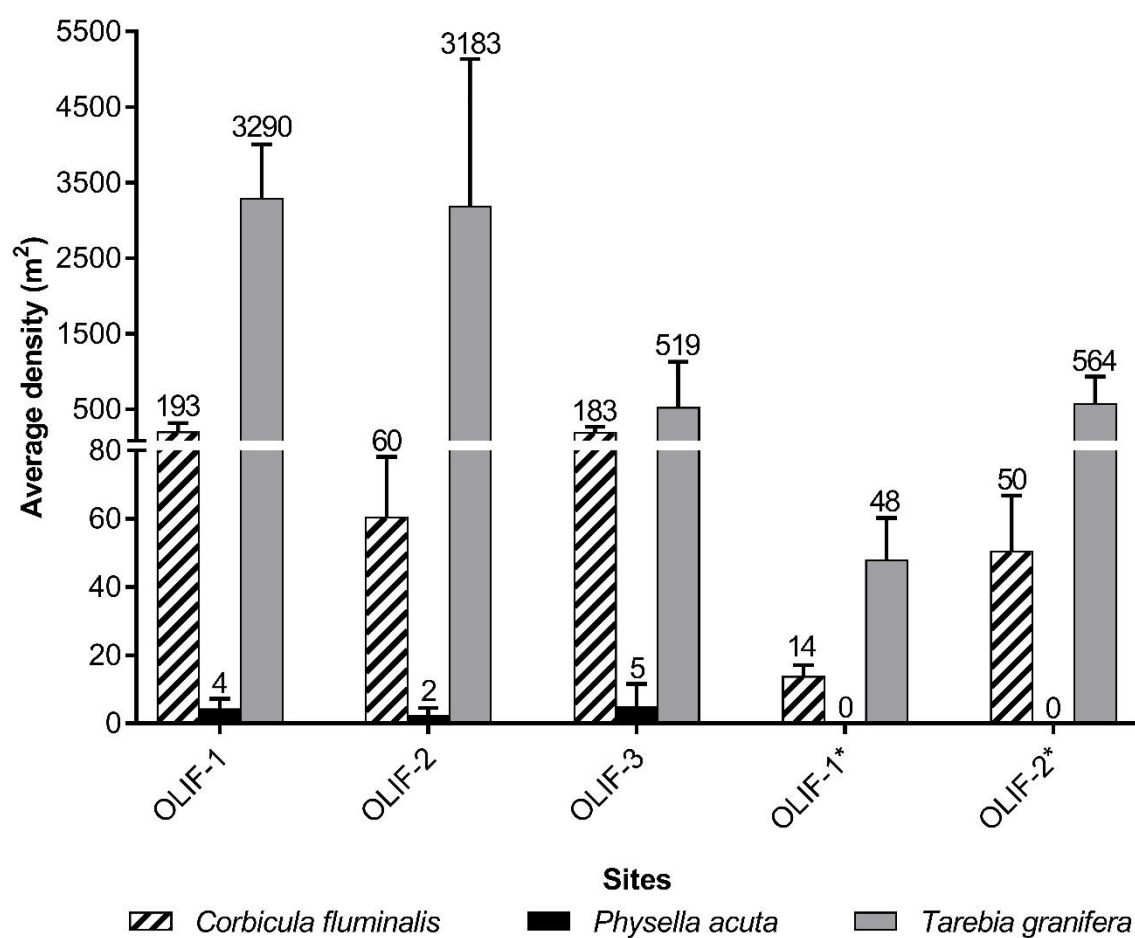


Figure 3.6: Average density ($\pm$ SD) of mollusc species sampled from the three sites on the Olifants River inside the Kruger National Park between September to October 2020 and April to May 2021. * Indicates sites sampled in 2021.

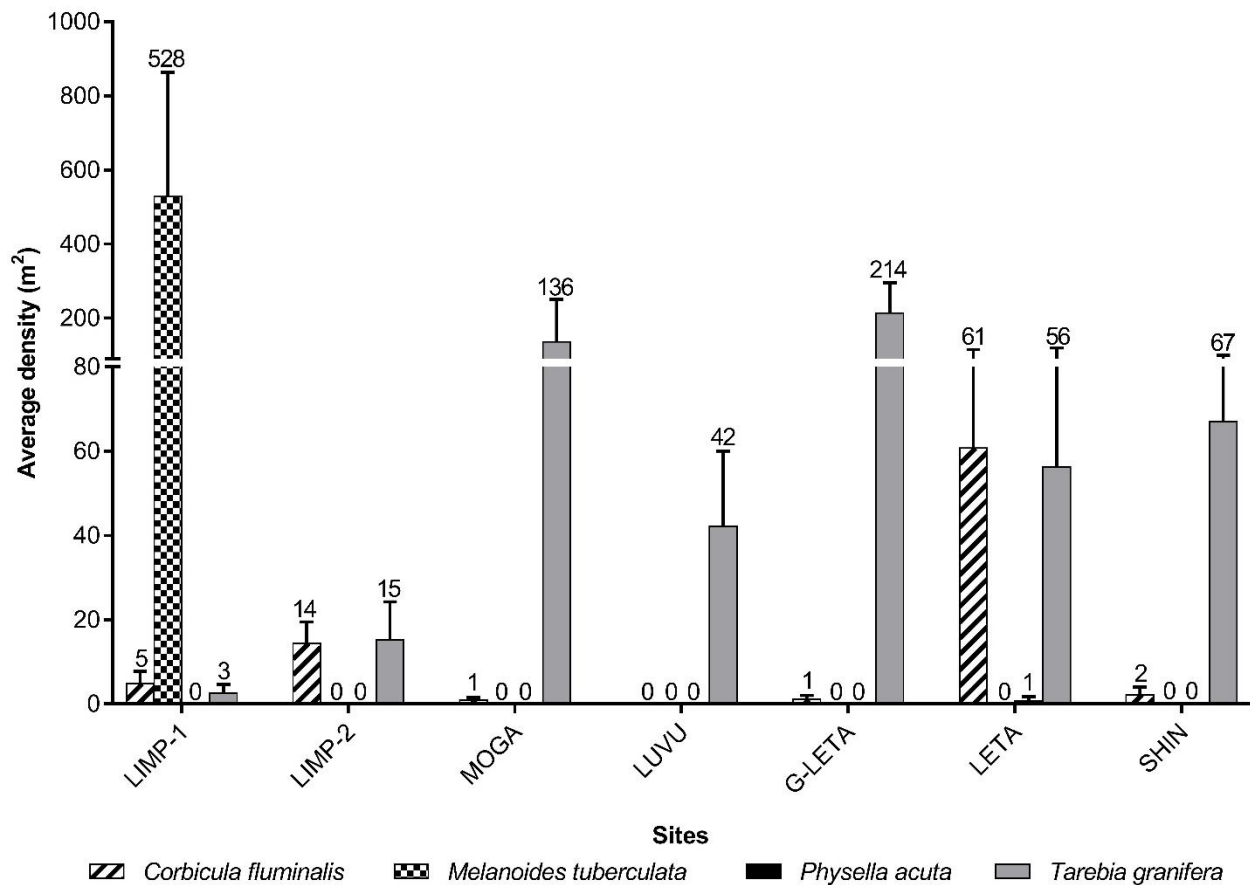


Figure 3.7: Average density ($\pm$ SD) of mollusc species sampled from the seven sites on the Limpopo River system between April to May 2021.

Kruskal-Wallis non-parametric tests (Table 3.4) indicated significant differences between snail species densities at the Phongolo River ($P = 0.0097$, $X^2 = 9.37$), Olifants River ($P = 0.0002$, $X^2 = 10.84$) and Limpopo River system ($P = 0.0017$, $X^2 = 15.16$). Dunn's post hoc test indicated this was as a result of significantly higher densities of *T. granifera* compared to *M. tuberculata* ($P = 0.016$) and *P. acuta* ($P = 0.049$) in the Phongolo River. In the Olifants River, this was as a result of significantly higher densities of *T. granifera* compared to *P. acuta* ($P = 0.002$) densities. From the other sites on the Limpopo River system, these differences were again as a result of significantly higher densities of *T. granifera* compared to *M. tuberculata* ($P = 0.006$) and *P. acuta* ($P = 0.001$) densities. There were no significant differences found between *T. granifera* and *C. fluminalis* densities.

Table 3.4: Kruskal-Wallis and Dunn's post hoc test of pairwise differences between the densities of *Tarebia granifera* and other mollusc species sampled on the three river systems. * Indicates significant differences ($p < 0.05$).

River	Kruskal-Wallis test		<i>Tarebia granifera</i> vs.	Dunn's post hoc test
Phongolo River	P = 0.0097*	$X^2 = 9.37$	<i>Corbicula fluminalis</i>	P = 0.098
			<i>Melanoides tuberculata</i>	P = 0.016*
			<i>Physella acuta</i>	P = 0.049*
Olifants River	P = 0.0002*	$X^2 = 10.84$	<i>Corbicula fluminalis</i>	P = 0.458
			<i>Physella acuta</i>	P = 0.002*
Limpopo River system	P = 0.0017*	$X^2 = 15.16$	<i>Corbicula fluminalis</i>	P = 0.230
			<i>Melanoides tuberculata</i>	P = 0.006*
			<i>Physella acuta</i>	P = 0.001*

Melanoides tuberculata were only sampled from two sites, PHON-1 on the Phongolo River and LIMP-1 on the Limpopo River. The PHON-1 population were not measured. The highest contributions to the LIMP-1 *M. tuberculata* population were from mature adults (6.25 – 12.64 mm) and adults (2.33 – 6.24 mm) (52.73 and 25.88% respectively) while older adults (> 12.64 mm) and juveniles (< 2.33 mm) contributed the least (21.25 and 0.14% respectively). Older adult *M. tuberculata* sampled from the LIMP-1 site had an average density of 112 individuals/m², their shells ranged from 12.65 to 34 mm in length and had an average length of 16.26 mm (± 3.82).

3.4 Discussion

Tarebia granifera has successfully invaded aquatic ecosystems across several continents as well as numerous water bodies in South Africa during the past two decades since it has been first reported in the country. The ecological drivers behind the successful spread of *T. granifera* have not been established thus far and shifts in the density and population size patterns of this invader, as well as its management status, are understudied (Raw *et al.*, 2013; Makherana *et al.*, 2022a). A better understanding of the population dynamics of this invader can help predict how they will react towards environmental changes which can lead to the development of management and control strategies (Burlakova *et al.*, 2010; Miranda *et al.*, 2011b; Makherana *et al.*, 2022a). This study investigated the distribution, densities and population size structures of *T. granifera* and other mollusc species from the Limpopo River system and Phongolo River. Research questions included whether *T. granifera* density is influenced by water quality variables and if native snail diversity and distribution are influenced by high densities of *T. granifera*. Results indicated no significant correlations between *T. granifera* density and any of the tested water quality variables and that the dominance of *T. granifera* at selected sites likely has a negative effect on native snail distribution and density.

3.4.1 *Tarebia granifera* density

Studies on the changes in *T. granifera* population densities have been contradictory as several studies reported maximum densities sampled at different times of the year (Appleton *et al.*, 2009; López-López *et al.*, 2009; Miranda *et al.*, 2011b). Miranda *et al.* (2011b) found that *T. granifera* density did not seem to undergo seasonal patterns in an estuarine environment, their study found that snail density was not significant in terms of the season at Catalina Bay, but that differences in *T. granifera* density was significant between locations during the wet season. In contrast, Makherana *et al.* (2022a) reported significant differences in *T. granifera* densities across seasons from a freshwater environment in Nandoni Dam, but no significant differences were found between their sites. The results from the present study also indicated differences in *T. granifera* density between seasons, maximum densities were recorded on the Limpopo River system during spring (September and October) after the winter season, while densities were much lower during autumn (April and May) after the summer season. Similarly, López-López *et al.* (2009) found that *T. granifera* populations from the Tuxpam and Tecolutla rivers (U.S.A) had pronounced depletions in snail density during the heavy rainy season (summer), were after their populations responded quickly during the dry season (winter) when nutrients increased food availability. Ecosystems sampled by de Kock and Wolmarans (2009) indicated that water velocity and rainfall played a significant role in the presence of the native thiarid species, *M. tuberculata*. It is thus not only important to note the time of the year, but also to consider the rainfall patterns prior to and during a survey (de Kock & Wolmarans, 1998), as rainfall play an important role in the densities and diversity of snail species (de Kock *et al.*, 2002; Wolmarans & de Kock, 2006).

The high densities of *T. granifera* recorded at selected sites during the present study correspond with those of previous studies in South Africa with densities of over 1 000 individuals/m² (Appleton and Nadasan, 2002; Appleton *et al.*, 2009; Miranda *et al.*, 2011a, 2011b). While the lower densities found during the present study at selected sites correspond with the lower densities reported by Makherana *et al.* (2022a) in the Nandoni Reservoir, South Africa. Nevertheless, even the low densities recorded during the present study were usually much higher than the densities reported for *T. granifera* (18 to 193 individuals/m²) in its native ranges (Dudgeon, 1980; Appleton & Nadasan, 2002). The lower densities found during the present study may be explained by population crashes that have been observed to occur in *T. granifera* populations due to environmental disturbances such as drought, flooding, food availability and increased salinity (Appleton *et al.*, 2009; López-López *et al.*, 2009; Miranda *et al.*, 2011b; Perissinotto *et al.*, 2014). The low population densities on the Limpopo River may indicate an early invasion period or a low carrying capacity from the system at the time of sampling (Makherana *et al.*, 2022a). However,

conclusions regarding such population density changes cannot be explained in the present study due to a lack of regular sampling over a longer period of time.

It has also been proposed that the high densities reached by *T. granifera* populations potentially minimize the probability of eradication due to environmental disturbances such as drought or increased salinity (Ben-Ami & Heller, 2005; Miranda *et al.*, 2011b). Miranda *et al.* (2011b) reported that one adult *T. granifera* (18 to 20 mm) can have an average of up to 48.6 unborn juveniles in its brood pouch and larger adults (20 to 30 mm) were found with an average of 158 unborn juveniles in their brood pouch. This suggests that only a single snail is needed to start a new population, this also gives some insight into how rapidly these invaders can reach high population densities even after population crashes due to environmental disturbances (Miranda *et al.*, 2011b).

Previous studies reported differences in *T. granifera* density due to differences in water pH, conductivity, and TDS (total dissolved solids) variables (Jones *et al.*, 2017; Makherana *et al.*, 2022a). Miranda *et al.* (2011b) suggested that the invasive snail demonstrated increased abundance as a response to heightened environmental stress. Jones *et al.* (2017) found a strong association between conductivity and abundance of *T. granifera* in the Nseleni River (South Africa) and Makherana *et al.* (2022a) reported significant differences in *T. granifera* density in the Nandoni Reservoir (South Africa) due to pH, conductivity, and TDS water quality variables. However, this does not seem to be the case in the present study as no significant correlations were found between *T. granifera* density and water quality variables in any of the sampled river systems. Miranda *et al.* (2011b) also found that in comparison with other snails, *T. granifera* was the least associated with any of the water quality variables and was the most dominant and widely distributed species from all study areas. Rather it seems increased conductivity and TDS may be as a result of the *T. granifera* ecology and biology, as this could likely be attributed to their movement behaviour in and through sediments (Appleton *et al.*, 2009) leading to the resuspension of contents from the sediments, for instance, salts and nutrients, and dissolve it back into the water column (Jolly *et al.*, 2008). High densities of benthic gastropods are known to cause sediment and microphytobenthos resuspension (Jensen & Siegismund, 1980; Hunt *et al.*, 1987; Morrissey, 1988; Andersen, 2001; Kelaher *et al.*, 2003; Orvain *et al.*, 2004).

Appleton *et al.* (2009) also found that some *T. granifera* populations from KwaZulu-Natal declined in density by as much as 95% every few years. This decline was attributed to low birth rates during the spring/summer season however, densities quickly started to build up again in only a few months (Appleton *et al.*, 2009). Makherana *et al.* (2022a) reported higher densities (368 individuals/m²) of *T. granifera* during summer and lower densities (104 individuals/m²) during autumn. According to Makherana *et al.* (2022a), the differences in density were a result of low

conductivity during autumn and winter and high conductivity and TDS during spring and summer. Results from the present study also indicated differences in *T. granifera* density between seasons, the Olifants River were sampled during spring (September and October) and autumn (April and May). The highest snail densities of *T. granifera* were recorded during spring while the densities decreased by 86.87% during autumn. This reduction in snail density during autumn could be ascribed to heavy rain/seasonal change and increased water velocity during the wet season (summer), as no significant correlations were found between *T. granifera* density and water quality variables. *Tarebia granifera* densities may quickly increase again during the dry season (winter-spring) when nutrients, and thus food availability increase (Appleton *et al.*, 2009; López-López *et al.*, 2009). This may also explain why higher densities of *T. granifera* were present in the Phongolo River during winter-spring (August and September) compared to sites sampled on the Limpopo River system during autumn (April and May).

3.4.2 *Tarebia granifera* population size structures

From all sites sampled during the present study, most *T. granifera* populations seemed to follow a trend of higher new-born recruitment during spring and summer while fewer new-born snails were recorded during autumn and winter months. Appleton and Nadasan (2002) also mentioned that there seems to be a peak in *T. granifera* birth during the summer months when it is warmer. *Tarebia granifera* have adapted to minimize mortality of early life stages during adverse events, different sizes of unborn snails have been found in the brood pouches of adult snails, suggesting that these unborn snails may be retained during unfavourable conditions (Miranda *et al.*, 2011b). Larger individuals of *T. granifera* have also been found to have a higher tolerance to desiccation and high salinity during dry events, enabling them to survive until more favourable conditions are reached (Chanotis *et al.*, 1980c; Facon *et al.*, 2004; Miranda *et al.*, 2011b). Interestingly, Miranda *et al.* (2011b) reported that some *T. granifera* populations seem to give birth all year round. This suggests that the birth rate may not be season dependent but rather depend on environmental variables such as water depth, wet or dry conditions, salinity, temperature and the availability of food sources (López-López *et al.*, 2009; Miranda *et al.*, 2011a, 2011b).

A higher proportion of adult and mature adult *T. granifera* was found in the Phongolo River, indicative of a decreased birth rate. The lower proportion of new-born snails from the Phongolo population might be explained by seasonal environmental changes as sampling occurred during winter-spring (dry season), the population will most likely continue to grow due to the higher number of adult snails that can reproduce during spring/summer when environmental conditions become more favourable (Appleton *et al.*, 2009). Miranda *et al.* (2011b) reported that *T. granifera* populations from Catalina Bay quickly re-established when favourable conditions returned after a period of desiccation in 2009, the adult population gave birth to large numbers of new-born snails

and by the end of 2010, the population had recovered in structure and density. The number of unborn snails in the brood pouch increased during times of environmental stress (increased salinity or desiccation) as well as during times of population recovery when environmental conditions became more favourable (Miranda *et al.*, 2011b). This suggests that *T. granifera* responds to environmental variability by increasing reproductivity and consequently speeding up population recovery (Miranda *et al.*, 2011b).

From the Limpopo River system, the highest contributions of new-born *T. granifera* were recorded from the OLIF-2 and OLIF-3 populations on the Olifants River in 2020 during spring (September to October). In contrast to these populations, the OLIF-1 population had low contributions of new-born snails during spring while the highest contribution of the population was from adults. During the 2021 survey (autumn), the new-born age group contributed the least to the OLIF-2 population indicating a reduction in birth during the survey, while the increase of the juvenile age group corresponded with the high contribution of new-borns from the previous season. The much lower average density recorded during the 2021 survey and the decline in the new-born age group may indicate that the population is in a state of recovery. Although the populations from the Limpopo River had no contributions from new-born snails, adults made up the largest part of the sampled populations. A similar trend in population size structure was found in the *T. granifera* populations from the Letaba River (LETA) and Shingwedzi River (SHIN). These sites were also sampled during autumn and the low contributions of new-born snails may be due to environmental changes (dry periods and lower temperatures) causing low reproduction in adults during autumn and winter months. Even though sampling occurred during autumn, higher contributions of new-born snails compared to other sites sampled during autumn occurred at the Mogalakwena, Luvuvhu, Shingwedzi and Great Letaba sites while all other sites had lower new-born contributions during autumn. This agrees with the statements of Miranda *et al.* (2011b) that *T. granifera* reproduction corresponds to environmental factors rather than the season and it seems that if environmental factors are favourable the snail will reproduce regardless of the season. The present study found similar results to those reported by Miranda *et al.* (2011a), with higher abundances of smaller size classes (< 10 mm). This is especially of concern as previous studies have indicated that juvenile snails can have higher impacts on food resources than larger adult snails as juvenile snails consumed more food by mass than adult snails (Boland *et al.*, 2008; Tamburi & Martín, 2009; Miranda *et al.*, 2011a).

The average birth rate of 1.89 new-born *T. granifera* per day from the present study corresponds with the reports that adults can give birth to one new-born snail every 12 hours (Abbott, 1952; Chaniotis *et al.*, 1980a; Sodeman, 1991; Appleton *et al.*, 2009). This ability to rapidly reproduce and the high environmental tolerance of mature adults play a key role in their success as an

invader and ensure the persistence of *T. granifera* within invaded habitats as repeated introductions are not essential for them to successfully colonise new areas (Appleton *et al.*, 2009; Miranda *et al.*, 2011b). From the present study, *T. granifera* samples from only three sites (LIMP-1, LIMP-2 and OLIF-1 during 2021) had no new-born snails, although all three of these sites had mature adults. The reason for no new-born snails at these sites is unclear and further information on the reproductive biology and population dynamics of *T. granifera* in South Africa are needed (Appleton *et al.*, 2009; Miranda *et al.*, 2011b). Juvenile *T. granifera* have also been reported to move over smaller spatial ranges than larger individuals (de la Vega *et al.*, 2003; Miranda *et al.*, 2011b) and it is possible that they occurred in areas that were not sampled during the survey.

3.4.3 Other mollusc diversity, density and community size structures

Invasive species often achieve densities higher than those found in their native areas and potentially have negative impacts on indigenous species (Carlton *et al.*, 1990; Hillebrand *et al.*, 2002; Gurevitch & Padilla, 2004; Pimentel *et al.*, 2005; Richardson & Pyšek, 2006; Prentis *et al.*, 2008; Gallardo *et al.*, 2016). This was evident from the present study, *T. granifera* made up 89% of all sampled snails and was the only snail species present at each of the 14 sites found to have snails. These findings are similar to that of previous studies where *T. granifera* have been reported to often be the dominant invertebrate species within invaded aquatic communities (Pointier *et al.*, 1998; Canete *et al.*, 2004; Pillay & Perissinotto, 2008; Appleton *et al.*, 2009; Weyl *et al.*, 2020). *Tarebia granifera* is therefore likely to have a negative impact on the community structures of native snail species, resulting in loss of biodiversity and declines in the abundance of native species (Hillebrand *et al.*, 2002; Appleton *et al.*, 2009; Jones *et al.*, 2017; Miranda *et al.*, 2011a; Miranda & Perissinotto, 2014).

While differences between densities of *T. granifera* and both *P. acuta* and *M. tuberculata* were significant, no significant differences were found between *T. granifera* and *C. fluminalis* densities. Apart from the PHON-2 and LUVU sites, the native *C. fluminalis* coexisted with *T. granifera* at all sites sampled during the present study, coexisting even at sites where *T. granifera* had the highest recorded densities. López-López *et al.* (2009) reported similar findings from the Tuxpam and Tecolutla rivers in the United States of America (U.S.A), where *T. granifera* and a *Corbicula* sp. (both invasive in the U.S.A.) coexisted and were both the dominant species sampled from invaded habitats while several native species and *M. tuberculata* (also invasive in the U.S.A) were missing or had limited distribution with low densities. These findings may be explained due to differences in the habitat distribution, feeding strategies and diet preferences of the different species (Tolley-Jordan & Owen, 2008; López-López *et al.*, 2009).

As mentioned in Chapter 1 (section 1.3), *T. granifera* has been reported to cause declines and extinctions in native snail populations from Cuba, Venezuela, Puerto Rico (Chaniotis *et al.*, 1980a, 1980b; Giboda *et al.*, 1997; Pointier & Giboda, 1999; Pointier & Jourdane, 2000; Karatayev *et al.*, 2009) as well as South Africa (de Kock & Wolmarans, 2008; Appleton *et al.*, 2009; Miranda *et al.*, 2011a, 2011b; Miranda & Perissinotto, 2012, 2014; Jones *et al.*, 2017; Kesner & Kumschick, 2018; Zengeya *et al.*, 2020). For this reason, they have been used in several ecosystems as biocontrol agents for *Schistosoma* host snails due to their ability to displace and outcompete other snail species outside of their native ranges (Prentice, 1983; Perez *et al.*, 1991; Sodeman, 1991; Pointier & Jourdane, 2000; Appleton *et al.*, 2009; Pointier *et al.*, 2010; Hewitt & Willingham, 2019).

The present study also found evidence of three native snail species that seem to have disappeared from their habitat. Empty shells (dead snails) of Planorbidae were sampled from the PHON-1 site, *B. tropicus* seem to have disappeared from the SHIN site and *B. forskalii* shells were collected from the MOGA site where *T. granifera* dominated. The native *M. tuberculata* was also missing from several sites during the present study. In addition, historical records of snail species from the sites sampled during the present study indicated that numerous species were missing from these sites such as *Biomphalaria* and *Bulinus* species that were missing from the Limpopo, Luvuvhu, Olifants, Letaba and Phongolo river sites (see Appendix A7 for more detailed information). Similarly, Miranda *et al.* (2011b) found that historically recorded snail species (*M. tuberculata* and *Bellamya capillata* Frauenfeld, 1865) from Lake Sibaya were absent during their surveys, and attributed this to the high densities of the invasive *T. granifera*. The absence of historically recorded and the low diversity of native snail species from the river systems sampled during the present study is of concern. Although this is quite likely linked to the ability of *T. granifera* to reach high densities and drive away other species (Appleton, 2003; de Kock & Wolmarans, 2007; Appleton *et al.*, 2009; Makherana *et al.*, 2022a), this requires further investigation before a definitive conclusion may be reached (de Kock *et al.*, 2002; de Kock & Wolmarans, 2008, 2009, 2010; Wolmarans & de Kock, 2006).

In terms of snail community size structures, the native *M. tuberculata* population from the Limpopo River (LIMP-1) followed the same trend as the *T. granifera* population from the site. *Melanoides tuberculata* also had fewer juveniles and more adults. Sampling occurred during autumn and the low contribution of juveniles is likely due to a reduction in birth during the autumn and winter months (dry season). This corresponds with the findings of Dudgeon (1986) that juvenile recruitment was higher during spring and summer. Dudgeon (1986) also mentioned that these juveniles grow rapidly after birth and become sexually mature before the following breeding season. However, in the United Arab Emirates, Ismail and Arif (1993) found two main periods of *M. tuberculata* juvenile recruitment in May (spring) and October (autumn). Their study also found

that some juveniles were born in April (spring), August (summer) and September (autumn). As *T. granifera* are able to reproduce throughout the year (Miranda *et al.*, 2011b), they may quickly become the dominant species and replace the native snails within this invaded ecosystem.

3.5 Conclusion

The purpose of this section of the study was to investigate the current distribution, densities, and population size structures of *T. granifera* at selected sites, in order to gain more information on how their populations persist and grow within these invaded habitats. Key interests included: whether there is a relationship between *T. granifera* density and selected water quality parameters and whether native snail diversity and distribution might be influenced by high densities of the invasive *T. granifera*. Results indicated that *T. granifera* were the dominant species of all molluscs sampled, the snail was also the only species to be present at each of the sites found to have molluscs. The densities reached by *T. granifera* were much higher than those of native molluscs and exceeded the densities reported from its native range. Higher population densities and higher new-born recruitment were observed during the sampling events in spring compared to autumn, likely as a result of more favourable environmental conditions during this period. In contradiction with previous reports, no significant correlations were found between *T. granifera* density and water quality variables in any of the sampled river systems. Furthermore, *T. granifera* density seem to have a negative effect on native snail density and diversity, especially since native snail diversity from the sampled systems was much lower than previously reported in the literature. At high densities *T. granifera* feeding may have further ecosystem wide implications on the food web dynamics of invaded ecosystems and further investigation is needed to determine how *T. granifera* affects native aquatic biota.

Chapter 4: The effects of *Tarebia granifera* on the aquatic community structures of benthic diatoms

4.1 Introduction

Biodiversity losses and declines of species from different trophic levels affect whole food webs as changes in any trophic level can lead to cascading effects in ecosystem function (Duffy *et al.*, 2007; Vaughn, 2010). Food web and species community structures influence how species interact with one another, both across and within different trophic levels (Downing & Leibold, 2002; Wojdak, 2005; Duffy *et al.*, 2007; Vaughn, 2010). Wojdak (2005) manipulated snail diversity (consumers), predation intensity and nutrient availability in a mesocosm experiment. Results from this study indicated that the effects of snail species richness on algae were dependent on the intensity of predation on the snails. Increases in nutrients led to higher primary production (algal biomass) and higher snail biomass, but predators caused a decline in snail biomass that indirectly led to more algal growth (Wojdak, 2005). Thus, snail grazing was responsible for structuring algal communities. Aquatic snails provide fundamental links between benthic productivity and higher trophic levels, at high densities these snails can have a significant impact on the availability of microphytobenthos e.g., diatoms, cyanobacteria and algae (Wojdak, 2005; Raw *et al.*, 2016). Aquatic snails can also alter nutrient cycles in aquatic ecosystems (Arnott & Vanni, 1996; Hall *et al.*, 2003; Carlsson *et al.*, 2004). Moslemi *et al.* (2012) indicated that at high densities, *Tarebia granifera* excreted more nitrogen. Changes in the availability of nutrients influence the growth and community structures of primary producers, e.g., plants and algae (Vanni, 2002), and may alter food web dynamics, with unknown implications on native aquatic consumers and higher trophic organisms (Moslemi *et al.*, 2012).

Previous studies indicated that *T. granifera* can exploit large numbers of microphytobenthos and detritus (March & Pringle, 2003; Miranda *et al.*, 2010; Miranda & Perissinotto, 2014) and their feeding impact can be much higher than that of other invertebrates (Miranda *et al.*, 2011a). At high densities, *T. granifera* may indirectly limit food availability which can result in decreased benthic biodiversity (Hill *et al.*, 2015; Miranda & Perissinotto, 2014) and disturbances in aquatic environmental function (Miranda *et al.*, 2011a, 2011b; Moslemi *et al.*, 2012; Raw *et al.*, 2013; Miranda & Perissinotto, 2014). As mentioned in Chapter 1, the ingestion rate of *T. granifera* is comparable to that of the golden apple snail (*Pomacea canaliculata*), one of the world's worst invasive species (Lowe *et al.*, 2000; Baker *et al.*, 2010; Miranda *et al.*, 2011a). Although *T. granifera* is a generalist feeder (Miranda *et al.*, 2011a), it may select certain groups of algae and diatoms over others, thus changing the biomass and community structure of certain algal groups within these communities (Armitage *et al.*, 2009). Generalist benthic grazers can reduce diatom

species abundance relative to other microalgal groups within benthic communities (Hagerthey *et al.*, 2002). Such losses in biodiversity include a decline in the abundance of common species and shifts in species dominance patterns (Hooper *et al.*, 2005).

Diatoms are a diverse group of microscopic autotrophic algae, their cell wall (frustule) is made up of silica and is composed of two valves that fit together (Mann & Droop, 1996; Rimet & Bouchez, 2012; Taylor & Cocquyt, 2019). Nitrogen and phosphorus are among the essential nutrients for diatom growth (Armitage *et al.*, 2009; Taylor & Cocquyt, 2019), whereas organic matter and nutrients have been found to affect diatom species diversity and richness (Patrick, 1961; Lange-Bertalot, 1979). Diatoms play a key role in aquatic ecosystems, as primary producers they are a food source for numerous heterotrophic species such as grazers and filter feeders (Goldfinch & Carman, 2000; James-Pirri *et al.*, 2001; Taylor & Cocquyt, 2019). Due to the high diversity and wide distribution as well as their rapid response to environmental changes, diatoms are excellent bioindicators and are used worldwide to assess the ecological quality of rivers (Rimet & Bouchez, 2012; Dalu & Froneman, 2016; Pandey *et al.*, 2017; Taylor & Cocquyt, 2019).

Aside from taxonomic classification, diatoms can be grouped according to functional groups (species with similar ecological/morphological characteristics), these functional groups include life forms, ecological guilds and size classes (Rimet & Bouchez, 2011; Viktória *et al.*, 2017; Kock, 2020). Benthic diatoms have evolved into several life forms (Mobile, Pedunculate, Colonial and Pioneers) to resist environmental pressures such as grazing, water flow fluctuations and the availability of nutrients (Rimet & Bouchez, 2011). Ecological guilds (High profile, Low profile and Motile) are groups of diatom species from the same environment with different adaptations to abiotic factors (Passy, 2007; Rimet & Bouchez, 2011). These life forms and ecological guilds provide information on diatom population structures and are often specific at the genus level (Rimet & Bouchez, 2011). Diatom functional groups such as ecological guilds, size classes, colonisation strategies and life forms occur more often across different aquatic ecosystems compared to specific taxa (Rimet & Bouchez, 2011) and are useful tools to determine environmental changes and stressors within aquatic ecosystems (Lukács *et al.*, 2018). Grouping species into ecological guilds can indicate stronger relationships with environmental pressures such as grazing, pollutants, water flow and nutrients compared to species data (Berthon *et al.*, 2011; Passy & Larson, 2011; Rimet & Bouchez, 2011, 2012). Diatom communities from lotic systems such as rivers utilize the same basic nutrients and are subject to the same environmental pressures e.g., grazing and water flow, but different diatom species have different adaptations to withstand such pressures (Passy, 2007). Currently, it is hard to predict how these complex and dynamic communities of diatoms will respond to high population densities of *T. granifera*.

This chapter aimed to determine the impacts of *T. granifera* on the benthic diatom community structures at selected *in situ* sites on the Limpopo River system and Phongolo River. Research questions included: 1) whether benthic diatom diversity and community structures may be influenced by *T. granifera* density and 2) whether changes in benthic diatom diversity and community structures could be related to differences in water quality variables or the differences in *T. granifera* densities between sites. These aims were achieved by collecting benthic diatoms and water samples from the same areas where *T. granifera* were sampled. The hypothesis for this chapter was that high densities of *T. granifera* in an aquatic community will result in a decrease in benthic diatom diversity, disrupting benthic diatom community structures and functionality.

4.2 Materials and methods

4.2.1 Diatom sampling and identification

Benthic diatom samples were collected from 10 of the 19 sites sampled for aquatic snails, mentioned in Chapter 3 (section 3.2.2): four sites on the Phongolo River (2017) and three sites each on the Olifants (2020 and 2021) and Limpopo (2021) rivers. Diatom samples were collected prior to the snail sampling and were collected from the same habitat (sediment surfaces) where snails were sampled from, except for the LIMP-3 site where snails were absent. The diatoms were sampled from the sediment with the use of a 50 ml syringe (Figure 4.1a) following the methods described by Taylor *et al.* (2007a). The syringe was lightly dragged over one meter (five X 20 cm drags) of the sediment surface with constant suction at a 45° angle, collecting diatoms from the top layer of the sediment. Samples were collected in triplicate at each site, and each of the replicates (3 X 50 ml samples) were fixed separately with 70% ethanol (with a final concentration not exceeding 20%) in 100 ml sample bottles. The three replicates (3 X 50 ml samples) from each of the sites on the Phongolo River in 2017, were fixed as a single sample per site, in 200 ml sample bottles.

Diatom samples were transported to the laboratory at the North-West University (NWU) and prepared with the “Hot Hydrochloric acid (HCl) and Potassium permanganate (KMnO₄)” method described by Taylor *et al.* (2007a). Samples were left for 24 hours to settle out, the clear supernatant was then decanted, and the remaining sample was shaken. Approximately two ml of the suspension was transferred to marked test tubes and treated with saturated KMnO₄ solution and left for 24 hours. Three ml of concentrated HCl (32%) was added to the sample and then boiled in a water bath at 90 °C until the solution became clear. After oxidation of the organic material, one drop of hydrogen peroxide (H₂O₂) was added to ensure all organic material was digested. Cooled samples were then washed by centrifuging with distilled water at 700 relative

centrifugal force (RCF) for 10 minutes. The supernatant was decanted and washing repeated four more times. The supernatant was decanted for the last time and 3 ml distilled water together with a drop of ammonium chloride (NH_4Cl ; 10%) was added to the samples to reduce aggregation. One drop of the sample was pipetted onto clean cover slides and left overnight to dry. Diatom-coated cover slides were mounted onto microscope slides using Pleurax (Refraction index, 1.73). A Nikon-80i compound light microscope (Figure 4.1b) equipped with a 100 x 1.4 N.A. oil immersion objective and a differential interference contrast (DIC) was used to identify and count diatoms. At least 400 diatom valves (individuals) were counted per slide and identified to species level where possible and grouped as centric or pennate diatoms using Taylor *et al.* (2007b) and the website: diatoms.org. Micrographs of select benthic diatoms found during the study are presented in Figure 4.1c-j.

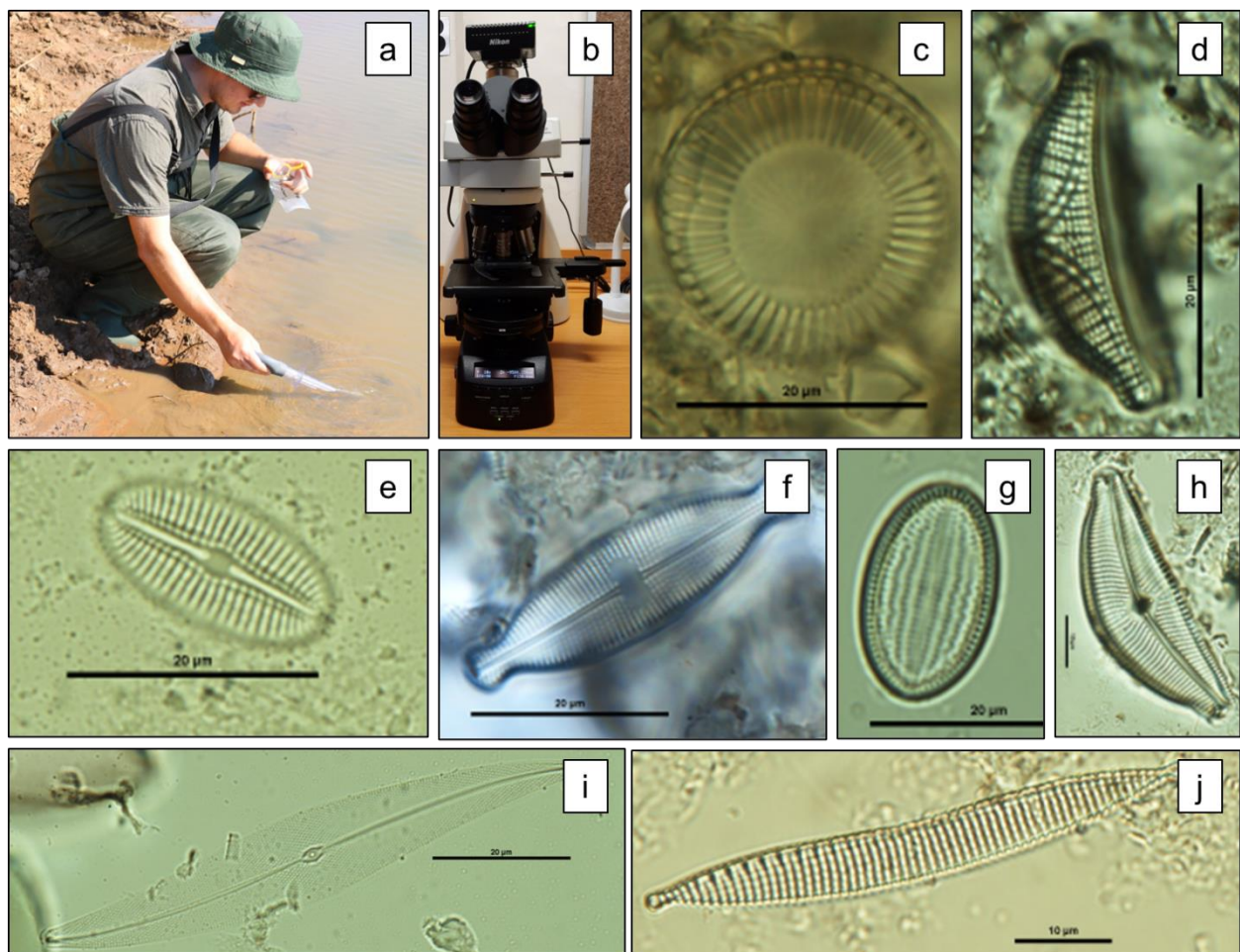


Figure 4.1: Methods used to collect and identify benthic diatoms from the selected sites sampled, a) sampling of benthic diatoms with a 50 ml syringe, b) diatoms were identified using a compound light microscope. Micrographs of some benthic diatom species found during the present study: c) *Cyclotella meneghiniana*, d) *Epithemia sorex*, e) *Diploneis elliptica*, f) *Mastogloia smithii*, g) *Cocconeis placentula*, h) *Cymbella tumida*, i) *Pleurosigma salinarum* and j) *Hantzschia distinctepunctata*.

4.2.2 Diatom functional group classification

Identified diatom species were divided into different functional groups according to taxonomic groups (Taylor *et al.*, 2007b), colonisation strategies (Stevenson, 1986; Ács & Kiss, 1993), size classes, life forms, ecological guilds (Rimet & Bouchez, 2011, 2012) and Combined Eco-Morphological Functional Groups (Viktória *et al.*, 2017). Centric taxa are cylindrical in shape and pennate diatoms are made up of four groups: Araphideae: taxa with no true raphe system and lack any significant motility, Biraphideae: taxa with a raphe on both valves and high motility, Raphidioideae: taxa with a raphe on both valves that has a short raphe system and Monoraphideae: taxa with a raphe on only one valve with a curved or flexed valve face (Taylor *et al.*, 2007b). Colonisation strategies consisted of Pioneer colonists (relative abundances of these species decrease during the final steps of colonisation), Intermediate colonists (have more stable relative abundances than other groups) and Late colonists (increase in abundance during the final steps of colonisation) (Stevenson, 1986; Ács & Kiss, 1993). Standardised size classes were assigned according to species biovolume: Size 1 consisting of taxa with biovolumes less than 99 μm^3 , Size 2 consisting of those between 100 and 299 μm^3 , Size 3 between 300 and 599 μm^3 , Size 4 between 600 and 1499 μm^3 and Size 5 greater than 1500 μm^3 (Rimet & Bouchez, 2012). Life forms included Pioneer (colonise bare substrate faster than other species), Colonial and Mobile (able to move as they have a raphe structure) (Rimet & Bouchez, 2011). Ecological guilds were made up of High profile (species with tall stature that are adapted to high nutrient concentrations and low water velocities), Low profile (species with short stature that are adapted to low nutrient concentrations and high-water velocity) and Motile (fast-moving species that can tolerate high nutrient concentrations but not high water velocity) guilds (Rimet & Bouchez, 2012). Combined Eco-Morphological Functional Groups (CEMFGs) were a combination of the ecological guilds (High profile – H, Low profile – L and Motile – M) and size classes (S1 – S5), creating 15 CEMFGs: HS1 – HS5, LS1 – LS5 and MS1 – MS5 (Viktória *et al.*, 2017).

4.2.3 Statistical analysis

Appleton and Nadasan (2002) reported *T. granifera* densities with means approaching 1000 m^2 as high and up to 2500 m^2 as exceptionally high, while Makherana *et al.* (2022a) reported density means of 104 m^2 as low. In its native habitat (Hong Kong, China), *T. granifera* was reported to have densities of 18 to 193 m^2 (Dudgeon, 1980; Appleton & Nadasan, 2002). For the present study, a density scale was developed based on the aforementioned reports to describe the effects of *T. granifera* density (from the selected sites) on benthic diatom community structures. The density scale is as follows: None (0 snails per m^2), Low (1 - 99 snails per m^2 ; similar to the lower end of densities in its native habitat), Medium (100 - 499 snails per m^2 ; similar to the higher end of densities in its native habitat and lower densities of invaded habitats in South Africa), High (500

- 1500 snails per m²; similar to reports of high densities from invaded habitats in South Africa) and Very high (> 1500 snails per m²; similar to extremely high densities reported from South Africa).

Primer version 7 software (Clarke & Gorley, 2006) was used to calculate univariate diversity indices. Margalef's index was used to calculate species richness, Pielou's evenness index was used to determine overall evenness and the Shannon-Wiener diversity index was used to determine overall diversity by integrating both species richness and equitability components (Clarke & Warwick, 1994). GraphPad Prism 7 was subsequently used to create graphical representations of the diatom species diversity indices and percentage (%) contribution of benthic diatom functional groups and CEMFGs. Prior to further analysis in Primer version 7, diatom data were square root ($\sqrt{}$) transformed to eliminate the effect of dominant taxa skewing the results (Malherbe *et al.*, 2010; de Necker *et al.*, 2016; 2022). A Bray-Curtis similarity matrix was used to create a dendrogram of the diatom community data from the selected sites. Sites were grouped for analysis according to *T. granifera* density (None, Low, Medium, High and Very high) as described above and according to the three different river systems (Phongolo, Olifants and Limpopo rivers) sampled.

These groupings were then tested for significance using the Analysis of Similarity (ANOSIM) procedure (Clarke & Gorley, 2006) using a preselected factor: "*T. granifera* density" or "river sampled", respectively. The ANOSIM provides two variables to interpret the tested significance: a Global R-value (from 0 to 1), R-values ≥ 0.5 indicate substantial differences whereas R-values < 0.5 indicate substantial similarity, while the significance level in percentage (%) indicate if the values are significant ($\leq 5\%$) or not ($> 5\%$) (Clarke & Warwick, 1994; Chapman and Underwood, 1999). Similarity percentage analysis (SIMPER) was then used to calculate the percentage (%) that benthic diatom communities contributed to similarities and pairwise dissimilarities between sites with different *T. granifera* densities and the different river systems sampled as factor groups, respectively.

Canoco version 5 (ter Braak & Šmilauer, 2012; Šmilauer & Lepš, 2014) was used to create an unconstrained Principal Component Analysis (PCA) to determine possible differences between benthic diatom communities from each of the sampled river sites with supplementary explanatory variables. These supplementary variables included: *T. granifera* density (None, Low, Medium, High and Very high), *T. granifera* age groups (Mature adults, Adults, Juveniles and New-borns) as described in Chapter 3 (section 3.2.2), and water quality variables. Prior to PCA, all supplementary explanatory data, except for pH, were log-transformed [$y = \log(x+1)$] to improve data normality (de Necker, 2019; de Necker *et al.*, 2022). Diatom community data (species and functional group abundance) were square root ($\sqrt{}$) transformed prior to the PCA to eliminate the

effect of dominant taxa/groups skewing the results (Malherbe *et al.*, 2010; de Necker *et al.*, 2016). The PCA creates a two-dimensional diagram of the data. Diatom species or groups plotted on the diagrams signify either similarities or dissimilarities between the sites based on the angle of the explanatory variables to each other. Variables bigger than 90° are negatively correlated while variables equal to or smaller than 90° are positively correlated (Šmilauer & Lepš, 2014).

4.3 Results

4.3.1 Diatom species community structures

A total of 118 benthic diatom species were identified from the ten selected sites, with 35 species collected from the Phongolo River (2017), 79 from the Olifants River (2020 and 2021) and 59 from the Limpopo River (2021). The highest number of species for the Olifants River were sampled during the 2020 survey (54 species), while fewer species were present during the 2021 survey (48 species). Appendix B1 to B3 summarises the diatom species abundances as well as different functional groups assigned to identified taxa of the sites sampled on the three rivers, respectively.

The Phongolo River was classified as having High densities of *T. granifera* (average 699 individuals/m² across all sites). Diatom species that were unique to this system included *Aulacoseira granulata*, *Capartogramma crucicula*, *Fragilaria tenera*, *Navicula erifuga*, *Navicula rostellata*, *Nitzschia siliqua* and *Rhopalodia gibberula*.

The Olifants River surveyed in 2020 was classified as having Very high densities of *T. granifera* (average 2 331 individuals/m² across all sites). Diatoms unique the Olifants River (2020), included *Amphora fontinalis*, *Amphora montana*, *Amphora pediculus*, *Caloneis hyaline*, *Caloneis silicula*, *Diploneis zanzibarica*, *Epi. sorex*, *Gomphonema aff. gracile*, *Gomphonema exilissimum*, *Gomphonema gracile*, *Gomphonema insigne*, *Gomphonema parvulus*, *Gomphonema pseudoaugur*, *Gomphonema pumilum*, *Gomphonema venusta*, *Navicula symmetrica*, *Nitzschia dissipata* var. *media*, *Nitzschia lancettula*, *Planothidium engelbrechtii*, *Rhoicosphenia abbreviata*, *Rhopalodia gibba* and *Tryblionella gracilis*. During the 2021 survey to the Olifants River, the river was classified as having Medium densities of *T. granifera* (average 306 individuals/m² across all sites). Diatoms unique the Olifants River (2021), included *Amphora strigosa*, *Caloneis molaris*, *Cymbella kolbei*, *Cymbella neocistula*, *Cymbella turgidula*, *Diploneis subovalis*, *Discostella stelligera*, *Eolimna minima*, *Fragilaria ungeriana*, *Nitzschia linearis*, *Nitzschia pumila*, *Pinnularia borealis*, *Pinnularia divergens* and *Tryblionella calida*.

The Limpopo River was classified as having Low densities of *T. granifera* (average 6 individuals/m² across all sites). Diatoms unique to the Limpopo River, included *Asterionella formosa*, *Craticula accomoda*, *Craticula ambigua*, *Craticula cuspidata*, *Dip. elliptica*, *Fallacia monoculata*, *Hippodonta capitata*, *Navicula antonii*, *Navicula microcari*, *Nitzschia amphibia*, *Nitzschia desertorum*, *Nitzschia littorea*, *Sellaphora pupula*, *Simonsenia delognei*, *Stephanodiscus agassizensis*, *Surirella agusta*, *Surirella ovalis*, *Surirella tenera*, *Tryblionella coarctata* and *Tryblionella hungarica*.

Benthic diatom communities from the Phongolo River had an average lower number of taxa and species richness compared to those of the Limpopo River system (Figure 4.2). On the Phongolo River, the sites with Medium *T. granifera* density (PHON-1 and PHON-4) had the lowest average number of diatom taxa (13 and 11), species richness (2 and 1.67) and Shannon diversity (2.29 and 2.18), respectively. Whereas the sites with Very high (PHON-2) and High (PHON-3) *T. granifera* densities had a higher average number of diatom taxa present (15 and 18), species richness (2.34 and 2.84) and Shannon diversity (2.41 and 2.83), respectively. The PHON-1 and PHON-2 sites had equal evenness (0.89), the PHON-4 site had higher evenness (0.91), while the PHON-3 site had the highest evenness (0.98) of all diatom communities across all sites.

The diatom communities from the three sites sampled on the Olifants River in 2020 had an average lower number of taxa and species richness compared to diatom communities sampled from the Olifants River in 2021 and Limpopo River, except for the LIMP-3 site that had the lowest average number of taxa (15.33) and species richness (2.39) of all sites sampled in the Limpopo River system. On the Olifants River (2020) the OLIF-1 and OLIF-2 sites that had a Very high *T. granifera* density, had the lowest average number of diatom taxa (15.67 and 16.33) and species richness (2.5 and 2.6, respectively). While the third site on the Olifants River (OLIF-3) with High *T. granifera* density had a higher average number of taxa (17.67) and species richness (2.78). In contrast, the OLIF-1 and OLIF-2 sites had greater evenness (0.88 and 0.86) and Shannon diversity (2.41 and 2.4), respectively, compared to OLIF -3 site that had an average evenness and Shannon diversity of 0.78 and 2.24, respectively.

Diatom communities sampled from the two sites (OLIF-1 and OLIF-2) on the Olifants River in 2021, had a higher average number of taxa (19.33 and 17.67), species richness (3.05 and 2.76), evenness (0.89 and 0.94) and Shannon diversity (2.62 and 2.69), respectively, than those sampled in 2020. The OLIF-1 site, which had a Low *T. granifera* density in 2021, had a higher number of diatom taxa and higher species richness while the OLIF-2 site with High *T. granifera* density had higher evenness and Shannon diversity. From the Limpopo River, diatom communities from the two sites (LIMP-1 and LIMP-2) with Low *T. granifera* density had the highest average number of taxa (19 and 19.67) and species richness (2.98 and 3.11), respectively, in the

system, while the third site (LIMP-3) where *T. granifera* was absent, had a lower average number of taxa (15.33) and species richness (2.39). The LIMP-1 site had the lowest average evenness (0.82) of the Limpopo diatom communities, while the LIMP-2 and LIMP-3 sites had similar evenness (0.9 and 0.89), respectively. Shannon diversity was the highest for the LIMP-2 (2.66) diatom communities, while LIMP-1 (2.4) and LIMP-3 (2.43) were similar.

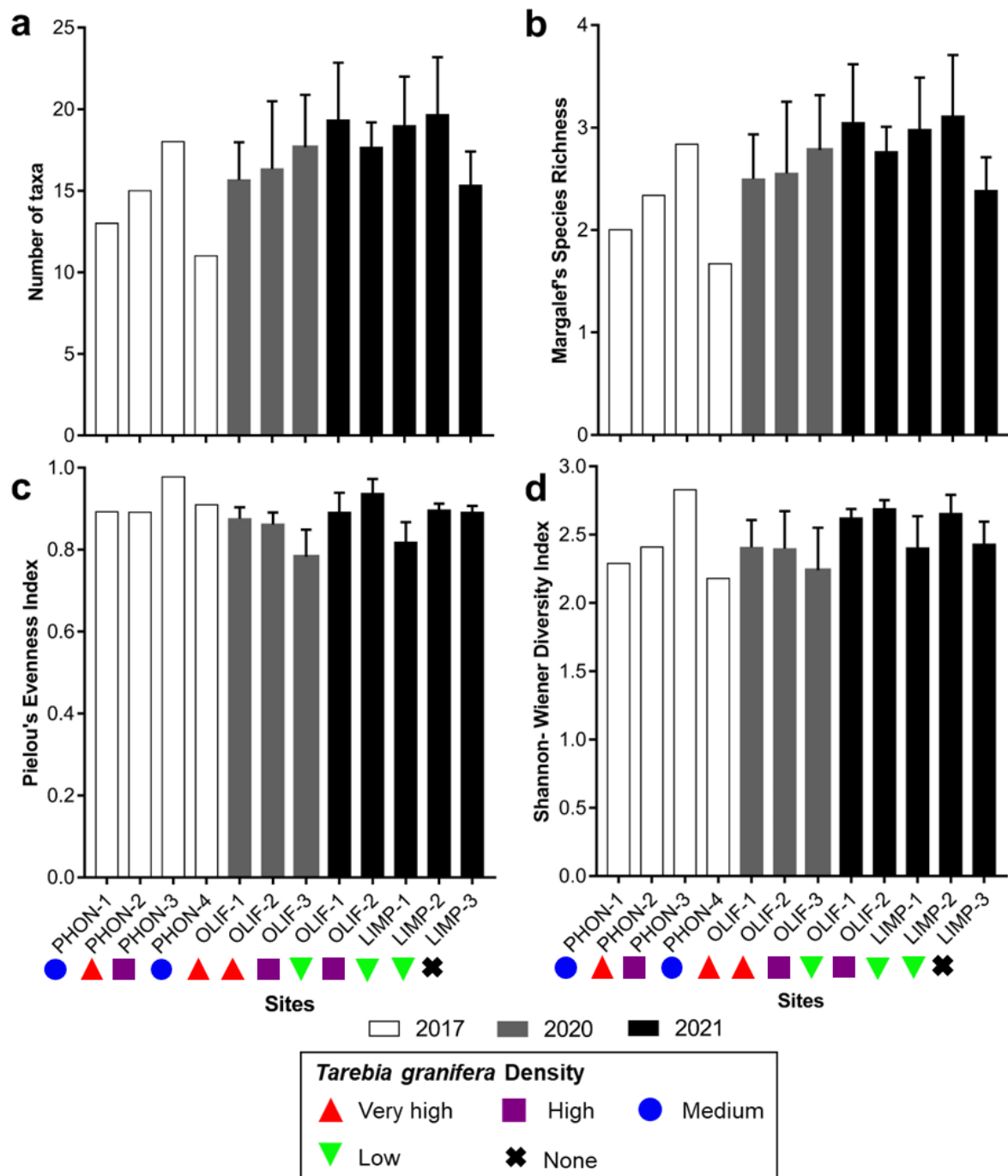


Figure 4.2: Mean values ($\pm$ SD) of diatom diversity indices sampled at the selected sites from 2017 to 2021: a) the total number of taxa, b) Margalef's Species Richness, c) Pielou's Evenness Index, d) Shannon- Wiener Diversity Index.

Sites with Low densities of *T. granifera* had higher abundances of *Craticula*, *Cymbella* and *Pleu. salinarum* species. Numerous diatom species were only present at Limpopo and OLIF-1 (2021) sites with Low densities of the invasive snail (refer to Appendix B1 to B3), including *Cal. molaris*, *Cym. kolbei*, *Cym. neocistula*, *Cym. turgidula*, *Encyonema minutum*, *Eol. minima*, *Fra. ungeriana*, *Luticola mutica*, four *Surirella* species, *Fal. monoculata*, *Ast. formosa*, *Cra. accomoda*, *Cra. ambigua*, *Cra. cuspidata*, *Dip. elliptica*, *Hip. capitata*, *Nav. antonii* and *Try. calida*. Diatom communities from sites with higher densities of *T. granifera* had the following dominant species: *Amphora*, *Cocconeis*, *Gomphonema*, *Nitzschia* and *Fragilaria*, while *Epi. sorex* and *Rhopalodia* sp. were only sampled from sites with Very high and High densities of *T. granifera*.

The dendrogram comparing diatom community structures of the selected sites revealed that communities separated between 18.84% to 50.32% similarity, indicating differences between river sites and diatom communities (Figure 4.3). Based on diatom species data, two groups formed at 18.84% similarity on the dendrogram, separating the diatom communities of the Phongolo River sites from those of the Limpopo River system sites. Within the Phongolo River grouping, two further groups formed at 35.97% similarity, separating the PHON-2 site with Very high *T. granifera* density from the other Phongolo sites with Medium (PHON-1 and PHON-4) and High (PHON-3) densities. The PHON-3 site subsequently separated at 41.1% similarity from the two sites with Medium *T. granifera* densities. Within the Limpopo River system grouping, two groups formed at 27.76% similarity. The diatom communities from sites with Low and no *T. granifera* sampled in 2021 (except OLIF-2) separated from the Olifants sites sampled in 2020 with Very high and High densities of *T. granifera*. Within the 2021 site grouping, two further groups formed at 31.92% similarity, separating the communities from the LIMP-1 site from the other 2021 sites. The LIMP-1 site was the only site where *Melanoides tuberculata* was sampled from all sites in the Limpopo River system, the site had densities of 528 *M. tuberculata*/m² and three *T. granifera*/m² (see Chapter 3, section 3.3.3).

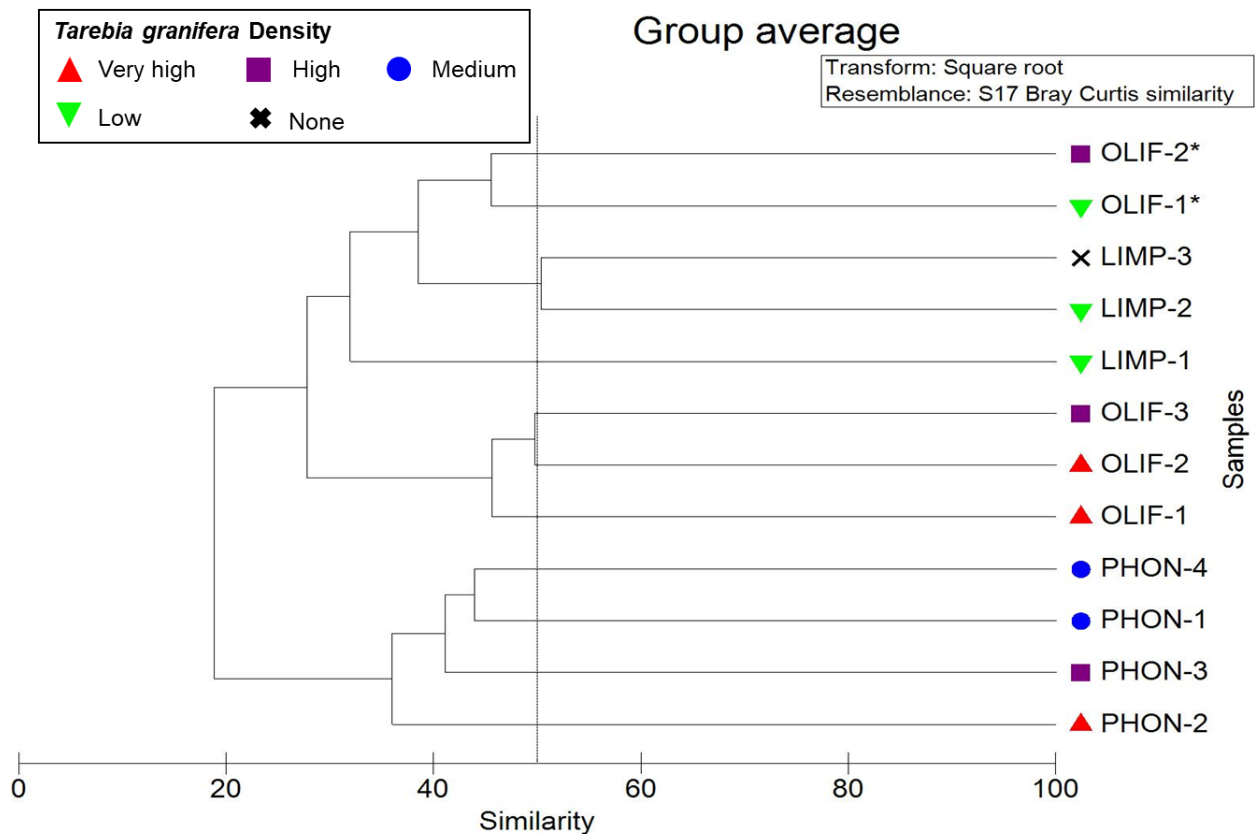


Figure 4.3: Dendrogram of diatom species sampled at the selected sites from 2017 to 2021. The dotted line indicates 50% similarity. The resemblance between sites is based on Bray-Curtis dissimilarity coefficients calculated from diatom community structures. Sites were grouped for analysis according to *Tarebia granifera* density (None, Low, Medium, High and Very high). * Indicates Olifants sites sampled in 2021.

The ANOSIM analysis based on the factor “*T. granifera* density” indicated that diatom communities were substantially similar ($R = 0.24$), and groupings were not significant (12.1%), indicating that *T. granifera* density as a factor had little discernible effect on the separation of diatom communities at sites. ANOSIM pairwise tests, however, indicated that diatom communities with Medium *T. granifera* densities present were substantially different ($R \geq 0.5$) from the diatom communities where Very high, High and Low *T. granifera* densities were present. Importantly, the diatom community where no *T. granifera* were present was substantially different ($R \geq 0.5$) from those diatom communities where *T. granifera* was present at all densities, although none of these groupings were significant ($> 5\%$). The following groupings were substantially similar ($R < 0.5$): Medium and High, Very high and Low, Very high and High, as well as High and Low *T. granifera* density but none of these groupings were significant ($> 5\%$). Based on the factor “river sampled”, the ANOSIM analysis revealed that communities were substantially different ($R = 0.86$), and groupings were significant (0.1%), indicating that the diatom communities from each of the sampled rivers were substantially different. ANOSIM pairwise tests indicated that the Phongolo

and Olifants as well as the Phongolo and Limpopo rivers had substantial differences ($R = 0.99$ and $R = 0.96$, respectively) while the Olifants and Limpopo rivers, although different ($R = 0.58$), were more similar. These groupings were all significant ($< 5\%$).

The SIMPER pairwise analysis, however, revealed substantial dissimilarities between diatom community structures based on *T. granifera* density groups (Appendix B5). Sites with Low densities and no *T. granifera*, although dissimilar (58.89%), were the most similar of all density factor groups. The diatom species contributing the most to dissimilarity between these groups were *Craticula halophila* (5.23%), *Tryblionella levidensis* (4.26%), *Pleu. Salinarum* (3.76%), *Nav. microcari* (3.34%) and *Navicula tripunctata* (3.25%), these species all had higher abundances at the site without *T. granifera*. Sites with Medium and no *T. granifera* densities were 86.97% dissimilar. The species that contributed the most to this dissimilarity were *Pleu. salinarum* (5.83%) and *Cra. halophila* (5.52%) that were only present at sites with no *T. granifera*, while *Coc. Placentula* (4.53%), *Nitzschia* sp. (4.5%) and *Navicula* sp. (4.49%) had higher abundances at the sites with Medium densities of the snail. Sites sampled with High densities and no *T. granifera* were 64.02% dissimilar. The species that had the highest contributions to this dissimilarity were *Pleu. Salinarum* (5.33%), *Nav. Microcari* (4.23%), *Cra. halophila* (4.1%) and *Nit. desertorum* (3.31%) that had higher abundances at the site without snails, while *Coc. placentula* (3.42%) dominated at sites with High densities of *T. granifera*.

Sites with Very high densities and no *T. granifera* were 79.79% dissimilar in diatom community structure. Diatom species contributing the most to this dissimilarity were *Pleu. Salinarum* (5.74%), *Nav. radiosa* (3.99%) and *Nav. microcari* (3.6%) that were only present at the site without snails, while *Gomphonema minutum* (3.44%) and *Coc. placentula* (3.27%) dominated at sites with Very high densities of *T. granifera*. Diatom communities with Medium and Low densities of the *T. granifera* were 84.74% dissimilar. The species with the highest contributions to this dissimilarity were *Craticula buderii* (4.87%) that were only found at sites with Low densities of the snail, while *Coc. placentula* (4.61%) dominated, and *Nitzschia* sp. (4.14%) and *Navicula* sp. (4.4%) were only found at sites with Medium densities of *T. granifera*. Communities with High and Low densities of *T. granifera* were 70.29% dissimilar in species composition. The diatom species contributing the most to this dissimilarity were *Cra. buderii* (3.48%) and *Pleu. salinarum* (3.13%) that had higher abundances at sites with Low densities, while *Coc. placentula* (3.19%) dominated at sites with High densities of *T. granifera*. Very high and Low density groups had a 75.51% dissimilarity in diatom community structure. The species with the highest contributions to this dissimilarity were *Coc. placentula* (3.49%) and *Gom. minutum* (3.48%) that dominated at sites with Very high densities, while *Nitzschia recta* (3.36%) and *Pleu. salinarum* (3.35%) were only found at sites with Low densities of *T. granifera*.

Medium and High density factor groups were 72.59% dissimilar in diatom species composition. Diatoms with the highest contributions to this dissimilarity were *Aul. granulata* (4.03%) that were only found at sites with Medium densities of *T. granifera*, while *Nitzschia* sp. (3.81%), *Navicula* sp. (3.77%), *Fragilaria ulna* var. *acus* (3.48%) and *Coc. placentula* (2.72%) dominated at sites with High densities of the invasive snail. Sites with Medium and Very high densities of the *T. granifera* were 77.09% dissimilar. The major diatom species that contributed to this dissimilarity were *Navicula* sp. (5.4%), *Gom. minutum* (4.2%), *Gomphonema* sp. (3.71%) and *Fra. ulna* var. *acus*. (3.68%) that had higher abundances at sites with Medium densities, while *Nitzschia* sp. (4.19%) dominated at sites with Very high densities. Diatom communities with Very high and High densities of *T. granifera* had 69.89% dissimilarity between groups. The diatom species that contributed the most to this dissimilarity were *Gom. minutum* (3.28%) and *Gomphonema affine* (3.23%) that had higher abundances at sites with Very high densities, *Nav. radiosa* (2.7%) was only present at sites with Very high densities, while *Aul. granulata* (2.67%) was only found at sites with High densities of *T. granifera*.

Based on the factor group “river sampled”, results from the SIMPER pairwise analysis revealed dissimilarities in diatom species composition between rivers sampled (Appendix B7). Diatom communities from the Phongolo (average High *T. granifera* density) and Olifants (average Very high *T. granifera* density) rivers were 80.29% dissimilar. The species contributing the most to this dissimilarity were *Nitzschia* sp. (3.99%), *Aul. granulata* (3.53%), *Navicula* sp. (3.4%) and *Nav. erifuga* (3.23%) that were only present in the Phongolo River, while *Han. distinctepunctata* (3.35%) and *Cra. buderii* (3.07%) were only found in the Olifants River. Phongolo (average High *T. granifera* density) and Limpopo (average Low *T. granifera* density) diatom communities had the highest dissimilarity (82.61%) in diatom species of the factor groups. Diatoms that contributed the most to this dissimilarity were *Pleu. salinarum* (5.24%) and *Cra. Buderii* (4.69%) that were only found in the Limpopo River, while *Coc. placentula* (3.87%) dominated and *Nitzschia* sp. (3.85%), *Aul. granulata* (3.4%), *Navicula* sp. (3.28%) and *Nav. erifuga* (3.11%) were only found in the Phongolo River. The Olifants (average Very high *T. granifera* density) and Limpopo (average Low *T. granifera* density) rivers were 71.17% dissimilar in diatom species composition. The species with the highest contribution to this dissimilarity were *Pleu. salinarum* (4.68%) and *Nav. tripunctata* (2.44%) that had higher abundances as well as *Epi. sorex* (2.28%) that were only found in the Limpopo River, while *Coc. placentula* (3.25%) and *Han. distinctepunctata* (2.84%) had higher abundances in the Olifants River.

The PCA tri-plot of the 25 best fitting benthic diatom species with regard to water quality, *T. granifera* density and *T. granifera* age groups, explain a total data variation of 40.62% (Figure 4.4). Three groups formed on the tri-plot based on differences in diatom communities as sites from similar river systems as well as sites with similar densities of *T. granifera* grouped together. The first axis (explaining 23.85%) indicated a separation of sites attributed to differences in diatom community structures from the Olifants and Phongolo sites compared to the Limpopo River sites. The Olifants River and Phongolo River sites were associated with higher densities of *T. granifera*, while diatom communities from the Limpopo River were not associated with high *T. granifera* density. *Navicula erifuga* were only found in the Phongolo River while *Nitzschia* sp., *Gomphonema* sp. and *Navicula* sp. had higher abundances at Phongolo sites. Olifants sites with Very high and High densities of *T. granifera* were associated with *Cocconeis*, several *Gomphonema* species and *Rho. gibba*. Limpopo sites with Low and no *T. granifera* densities were associated with *Nav. microcari*, *Nitzschia desertotum* and *Sel. pupula* that were only present at sites with Low densities of *T. granifera*, as well as *Pleu. salinarum* and *Cra. halophila* that were also associated with sites with Low and no densities of *T. granifera*. On the second axis (explaining 16.77%) Olifants sites separated from the other sites below the axis due to the higher levels of conductivity (EC) as well as several organic nutrients (PO_4^{3-} , NO_3^- and SO_4^{2-}), while higher water temperature was associated with the Phongolo sites and higher pH and Cl^- with Limpopo sites. Diatom communities from the Olifants River with higher densities of *T. granifera* were different from those of the Limpopo and Phongolo rivers with lower densities. The Mature adult age group was associated with the Phongolo sites, as these sites had the highest abundance of this age group compared to the other sites. The sites from the Olifants River were associated with higher numbers of Juveniles and New-borns, while sites on the Limpopo River were not associated with any *T. granifera* age groups due to the low densities of *T. granifera* sampled from these sites.

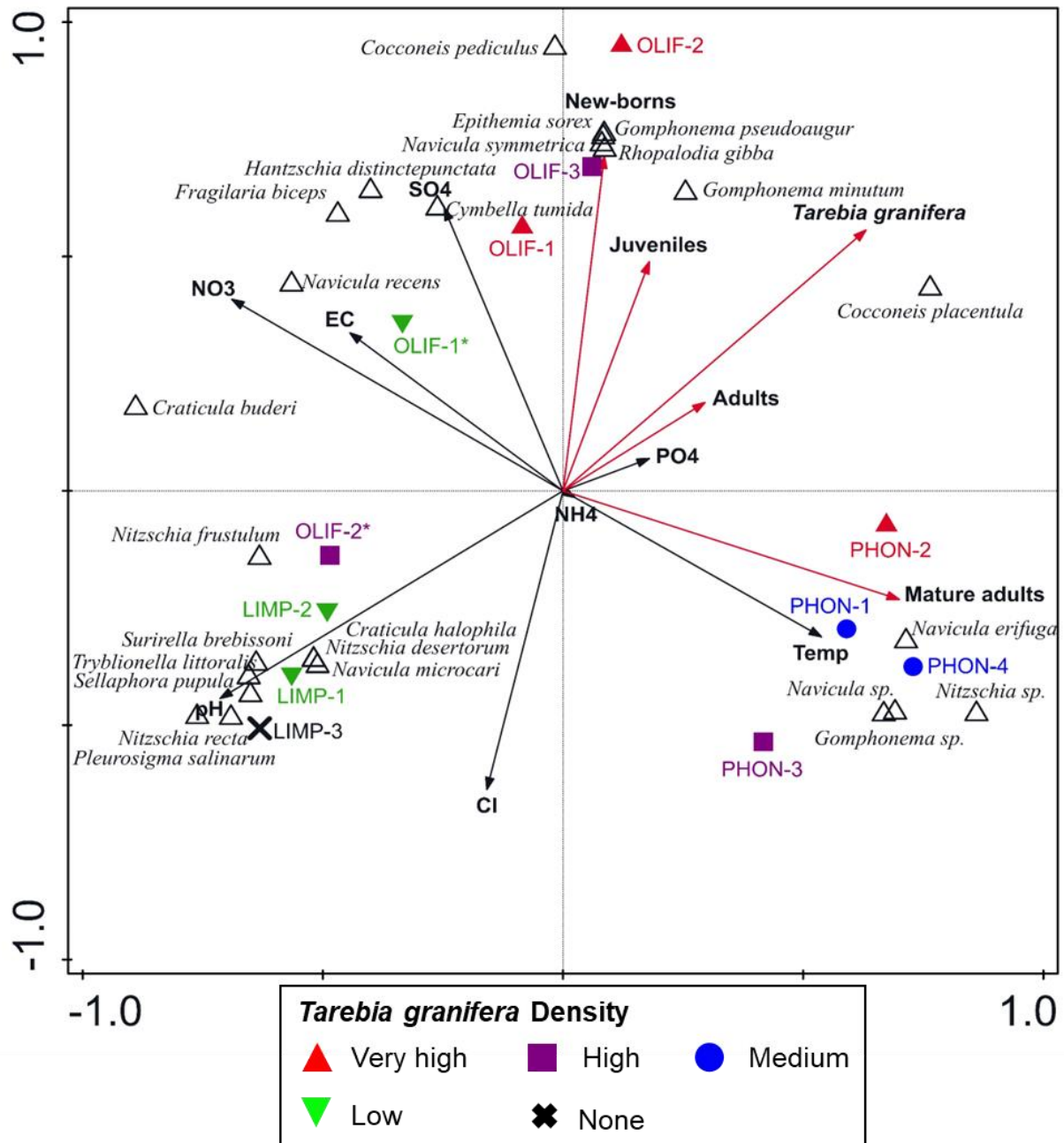


Figure 4.4: Principal Component Analysis (PCA) tri-plot of the 25 best fitting diatom species (triangles) sampled at the selected sites from 2017 to 2021 with water quality variables (black arrows) as well as *Tarebia granifera* density and age groups (red arrows) plotted as supplementary variables. The tri-plot explains 40.62% of the total data variation of which 23.85% is displayed on the first axis and 16.77% on the second axis. Sites were grouped according to *T. granifera* density (None, Low, Medium, High and Very high) and age groups (Mature adults, Adults, Juveniles and New-borns). * Indicates Olifants sites sampled in 2021.

4.3.2 Diatom functional group community structures

In terms of colonisation strategy, all sites were dominated by Intermediate colonists except for OLIF-2 (surveyed during 2020) where Pioneer colonists (49.02%) dominated (Figure 4.5a). Sites with Low densities and no *T. granifera* had higher abundances of Late colonists: OLIF-1 (13.64%) surveyed during 2021, LIMP-1 (26.32%), LIMP-2 (15.36%) and LIMP-3 (29.7%). In contrast, much lower abundances of Late colonists were present at sites with Very high densities: PHON-2 (3.75%), OLIF-1 (8.24%) and OLIF-2 (6.96%) surveyed during 2020, as well as sites with High densities of *T. granifera*: OLIF-3 (9.17%) and OLIF-2 (8.84%) surveyed during 2021. Sites with Very high densities of *T. granifera* also had the highest numbers of Pioneer colonists: PHON-2 (35%) and OLIF-2 (49.02%).

Biraphideae were the dominant taxonomic group from all sites sampled (Figure 4.5b). However, sites with Low densities and no *T. granifera* had even greater abundances of Biraphid diatoms: OLIF-1 (87.83%) surveyed during 2021, LIMP-1 (86.56%), LIMP-2 (87.45%) and LIMP-3 (96.06%). While sites with Very high densities: PHON-2 (61.25%), both OLIF-1 (73.7%) and OLIF-2 (74.55%) surveyed during 2020, as well as High densities of *T. granifera*: PHON-3 (82%), OLIF-3 (57.93%) and OLIF-2 (79.68%) surveyed during 2021, had lower abundances of Biraphideae. Sites with Very High: PHON-2 (15%), both OLIF-1 (23.24%) and OLIF-2 (23.81%) surveyed during 2020, High: PHON-3 (13.25%) and OLIF-3 (41.16%), and Medium: PHON-1 (22.25%) and PHON-4 (33.75%) densities of *T. granifera* had higher abundances of Monoraphideae. While sites with Low densities: OLIF-1 (6.21%) surveyed in 2021, LIMP-1 (4.9%), LIMP-2 (6.36%), and the site with no snails: LIMP-3 (3.86%) had much lower abundances of Monoraphid diatoms.

Small diatoms (size class 1 and 2) contributed the least, while large diatoms (size class 5) had the highest contribution of all size classes at all sites sampled (Figure 4.5c). Small diatoms (size class 2) had the highest abundances at sites with Low: OLIF-1 (9.48%) surveyed in 2021, LIMP-1 (16.76%), LIMP-2 (16.10%), no: LIMP-3 (14.03%) densities of *T. granifera*. While sites with Very high: PHON-2 (3.75%), both OLIF-1 (7.59%) and OLIF-2 (5.65%) surveyed during 2020, and High: PHON-3 (7.25%), OLIF-3 (2.4%) and Medium: PHON-4 (6.25%) densities had lower abundances of small diatoms. Sites with Low densities (LIMP-1 and LIMP-2) and no *T. granifera* present (LIMP-3) had diatom communities with more evenly distributed size classes, while sites with Very high: OLIF-2 (73.81%) surveyed in 2020, High: OLIF-3 (61.49%), and Medium: PHON-1 (58%) densities demonstrated a dominance of large diatoms (size class 5).

The Mobile life form was the dominant group of the three diatom life forms, contributing to more than 67% at all sites sampled (Figure 4.5d). The PHON- 3 site with High *T. granifera* density, and the LIMP-3 site without snails both only had Mobile diatom life forms (100%). Pioneer life forms did not contribute to any of the diatom communities. The Colonial life form had the highest abundances at the PHON-1 site with Medium *T. granifera* density (32%), PHON-2 with Very High *T. granifera* density (27%), and the OLIF-2 site (surveyed in 2021) with High *T. granifera* density (9%).

The Motile diatom ecological guild dominated across all sites sampled (Figure 4.5e). During the 2021 survey on the Olifants River, the High profile guild increased by 106.6% (from 23.33 to 48.20%) at the OLIF1 and 55.15% (from 11.46 to 17.78%) at the OLIF-2 sites compared to the 2020 survey. In contrast, the Low profile guild decreased by 77.78% (from 38.61 to 8.58%) at the OLIF-1 and 47.79% (from 28.56 to 14.34%) at the OLIF-2 sites from 2020 to 2021. *Tarebia granifera* densities also decreased by 98.54% at the OLIF-1 and by 82.28% at the OLIF-2 sites during 2021 survey compared to the 2020 survey (see Chapter 3, section 3.3.1). Sites with Low densities of *T. granifera* had lower abundances of Low profile ecological guilds compared to the sites with higher densities. Sites with higher densities of *T. granifera* had more than three times more Low profile guild species than sites with lower densities of the snail.

The HS1 and LS2 groups were the only two CEMFGs that did not contribute to any of the diatom communities from all sites sampled (Figure 4.5f). The remaining small-sized CEMFGs (HS2, LS1 and MS1) had higher abundances at sites with Low densities (OLIF-1 surveyed in 2021, LIMP-1 and LIMP-2) and no *T. granifera* (LIMP-3), compared to sites with higher densities of the snail. In contrast, LS5 and MS5 had higher abundances at sites with higher densities of *T. granifera* compared to sites with Low densities and no *T. granifera*, except for the HS5 group that dominated (40%) at the OLIF-1 site (surveyed in 2021) with a Low density of *T. granifera*. The LS5 group dominated at the PHON-1 (42%) site with Medium *T. granifera* density, as well as the OLIF-1 (37%) site surveyed in 2020 with Very high and OLIF-3 (34%) site with High *T. granifera* densities.

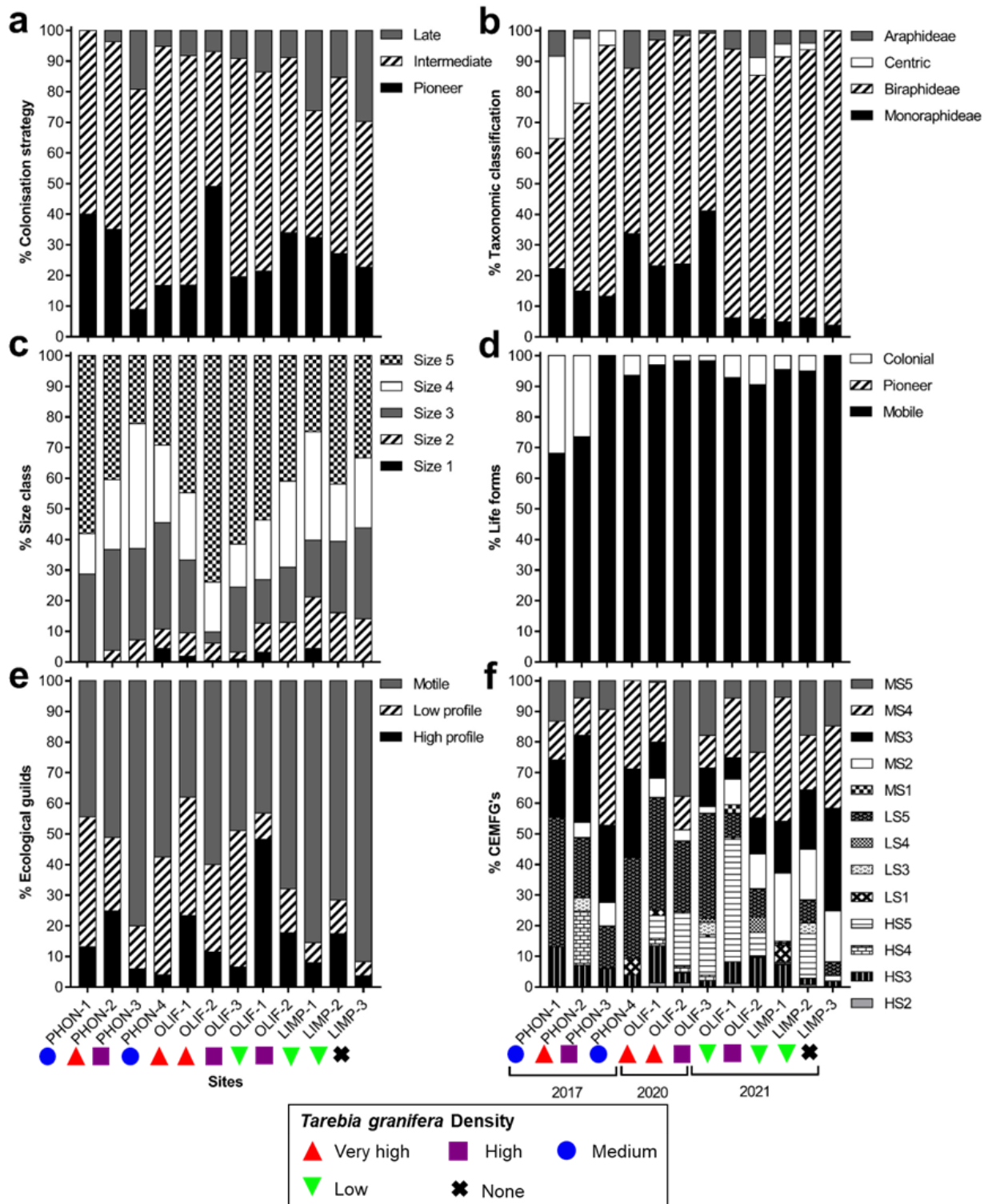


Figure 4.5: Percentage contribution (%) of diatom functional groups sampled at the selected sites from 2017 to 2021: a) colonisation strategy, b) taxonomic classification, c) size classes, d) life forms, e) ecological guilds, f) Combined Eco-Morphological Functional Groups (CEMFGs).

The dendrogram comparing diatom community functional group composition between the selected sites revealed that communities were very similar in terms of functional group composition, communities separated between 78.44 to 91.33% similarity (Figure 4.6). Two groups formed on the dendrogram at 78.44% similarity, separating the diatom communities of the sites sampled in 2021 (except PHON-3) with lower densities of *T. granifera* from the sites with higher densities of *T. granifera* sampled on the Phongolo (sampled in 2017) and Olifants (sampled in 2020) rivers. Diatoms from the Limpopo and OLIF-1 (sampled in 2021) sites with Low densities of *T. granifera*, shared more than 85% similarity in functional group composition with the PHON-3 and OLIF-2 (sampled in 2021) sites with High densities of *T. granifera*. The Phongolo River sites with Medium (PHON-1 and PHON-4) and Very high (PHON-2) densities of *T. granifera* separated at a lower (between 79.56 and 82.01%) similarity from the Olifants sites (sampled in 2020) with Very high (OLIF-1 and OLIF-2) and High (OLIF-3) densities of *T. granifera*.

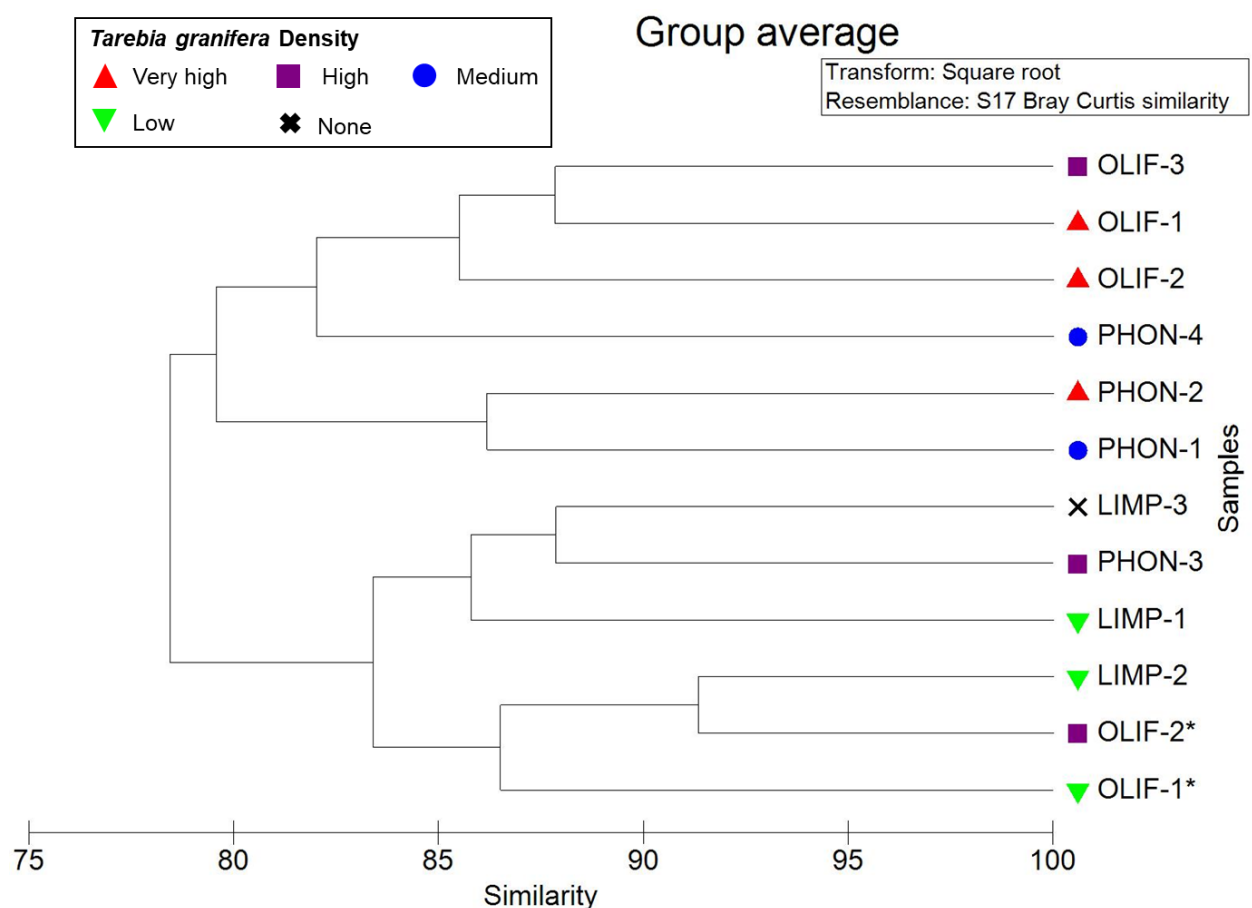


Figure 4.6: Dendrogram of diatom functional groups sampled at the selected sites from 2017 to 2021. The resemblance between sites is based on Bray-Curtis dissimilarity coefficients calculated from diatom functional group structures. Sites were grouped for analysis according to *Tarebia granifera* density (None, Low, Medium, High and Very high). * Indicates Olifants sites sampled in 2021.

The ANOSIM analysis based on the factor “*T. granifera* density” indicated that diatom communities were substantially similar ($R = 0.28$) in functional group composition, and groupings were not significant (8.2%), indicating that the factor “*T. granifera* density” had little discernible effect on the separation of diatom functional groups at sites. ANOSIM pairwise tests, however, indicated that diatom functional groups with Medium and Low, Medium and None, as well as Very high and None *T. granifera* densities present were substantially different ($R \geq 0.5$) in functional group composition, although none of these groupings were significant ($> 5\%$). The following groupings were substantially similar ($R < 0.5$) in functional group composition: Medium and Very high, Medium and High, Very high and High, Very high and Low, High and None, High and Low, as well as Low and None *T. granifera* density but none of these groupings, were significant ($> 5\%$). Based on the factor “river sampled”, the ANOSIM analysis revealed that diatom functional group communities were substantially different ($R = 0.5$), and groupings were significant (0.3%), indicating that the diatom communities from each of the sampled rivers were substantially different in diatom functional group composition. ANOSIM pairwise tests indicated that the Phongolo and Olifants rivers had substantial differences ($R = 0.55$) and groupings were significant (0.8%). While the Phongolo and Limpopo, as well as the Olifants and Limpopo rivers, had substantial similarities ($R = 0.46$ and $R = 0.49$, respectively). These groupings were significant (3.6%) for the Olifants and Limpopo grouping, but not significant (5.7%) for the Phongolo and Limpopo grouping.

The SIMPER pairwise analysis revealed low dissimilarities between diatom functional groups based on *T. granifera* density groups (Appendix B9). Sites with Low densities and no *T. granifera*, although dissimilar (15.56%), were the most similar to one another of all groups. Diatom functional groups contributing the most to this dissimilarity were HS5 (9.28%), High profile (8.61%), Colonial (7.38%) and Araphid (6.91%) diatoms that had higher abundances at sites with Low densities of the snail, while the MS3 (6.47%) group was more abundant at the site with no *T. granifera*. Sites with Medium and no *T. granifera* densities were the most dissimilar (26.8%) in diatom functional group composition of all density groups. The functional groups with the highest contributions to this dissimilarity were Late colonists (8.09%) and MS2 (7.67%) groups that had higher abundances at the site without *T. granifera*, while Low profile (7.94%), Colonial (7.68%) and LS5 (7.54%) groups had higher abundances at sites with Medium densities of *T. granifera*. Sites sampled with High densities and no *T. granifera* were 17.29% dissimilar. Diatom functional groups that had the highest contributions to this dissimilarity were Late colonists (5.68%) that had higher abundances at the site without *T. granifera*, while Low profile (7.44%), Monoraphideae (6.32%) and LS5 (5.98%) functional groups dominated at sites with High densities of *T. granifera*.

Groups with Very high densities and no *T. granifera* were 23.92% dissimilar. The functional groups contributing the most to this dissimilarity were Late colonists (6.11%) and MS3 (5.97%) groups that had higher abundances at the site without *T. granifera*, while Low profile (6.9%), LS5 (6.12%) and Colonial (5.57%) functional groups dominated at sites with Very high densities of *T. granifera*. Diatom functional group communities from sites with Medium and Low densities of the *T. granifera* were 24.37% dissimilar in functional group composition. The major functional groups contributing to the dissimilarity were MS2 (7.63%) and HS5 (6.63%) groups that were only found as well as Late colonists (6.09%) that had higher abundances at sites with Low densities of the snail, while LS5 (7.78%), Low profile (6.73%) and Monoraphideae (5.61%) dominated at sites with Medium densities of *T. granifera*. Diatom communities with High and Low densities of *T. granifera* were 17.03% dissimilar. Functional groups contributing the most to this dissimilarity were HS5 (7.47%) and High profile (5.25%) groups that had higher abundances at sites with Low densities, while LS5 (5.65%) and Monoraphideae (5.25%) dominated at sites with High densities of *T. granifera*. Very high and Low *T. granifera* density groups had a 19.64% dissimilarity, the major functional groups contributing to this dissimilarity were LS5 (6.97%), Low profile (6.09%), Monoraphideae (5.06%) and HS4 (4.96%) groups that had higher abundances at sites with Very high densities of *T. granifera*.

Medium and High density factor groups were 20.48% dissimilar. Diatom functional groups that contributed the most to this dissimilarity were of MS2 (5.98%), MS5 (5.7%) and Late colonists (5.51%) functional groups that had higher abundances at sites with High densities, while Colonial (6.74%) and Centric (6.15%) groups had higher abundances at sites with Medium densities of *T. granifera*. Sites with Medium and Very high densities of the *T. granifera* were 19.28% dissimilar and the major diatom functional groups that contributed to this dissimilarity were Centric (6.47%), MS3 (5.69%) and Colonial (5.58%) groups that had higher abundances at sites with Medium densities, while MS5 (6.66%), HS4 (5.94%) and HS5 (5.81%) groups dominated at sites with Very high densities. Diatom communities with Very high and High densities of *T. granifera* had a 17.67% dissimilarity between density groups. The functional groups that contributed the most to this dissimilarity were Colonial (5.3%), HS4 (4.84%) and HS5 (4.85%) groups that had higher abundances at sites with Very high densities, while MS3 (5.36%) and MS5 (6.33%) groups had higher abundances at sites with High densities of *T. granifera*.

Based on the factor group “river sampled”, results from the SIMPER pairwise analysis revealed low dissimilarities in diatom functional group composition between the rivers sampled (Appendix B11). Diatom communities from the Phongolo (average High *T. granifera* density across sites) and Olifants (average Very high *T. granifera* density across sites) rivers were 20.57% dissimilar in functional group composition. The functional groups contributing the most to this dissimilarity

were HS5 (9.11%) that were only found in the Olifants River, while Centric (6.42%), MS3 (5.58%) and Colonial (5.55%) groups had higher abundances in the Phongolo River. Phongolo (average High *T. granifera* density across sites) and Limpopo (average Low *T. granifera* density across sites) diatom communities had the highest dissimilarity (21.43%) of the factor groups. Diatom functional groups that contributed the most to this dissimilarity were MS2 (6.87%) and Late colonists (6.24%) that had higher abundances in the Limpopo River, while LS5 (7.15%), Low profile (6.05%) and Colonial (5.89%) functional groups were dominant in the Phongolo River. Although the dissimilarity in diatom communities between the Olifants (average Very high *T. granifera* density across sites) and Limpopo (average Low *T. granifera* density across sites) rivers were 19.21%, they were the most similar of the rivers sampled. The functional groups that contributed the most to this dissimilarity were LS5 (6.4%), HS5 (6.27%), Low profile (5.93%) and Monoraphideae (4.98%) that had higher abundances in the Olifants River, while the Limpopo River had higher abundances of MS3 (5.44%), Motile (4.86%) and MS2 (4.64%) diatoms.

The PCA tri-plot (Figure 4.7) comparing differences in diatom functional groups between sites with regard to water quality, *T. granifera* density and *T. granifera* age groups, explains a total variation of 57.83% in the data. Sites separated on the first axis (explaining 34.86%) due to differences in diatom functional groups at Olifants and Phongolo sites (except PHON-3 and OLIF-2 sampled in 2021) that were associated with higher densities of *T. granifera*, while Limpopo and OLIF-1 (sampled in 2021) sites were not associated with high *T. granifera* density. Sites with higher densities of *T. granifera* were associated with the following functional groups: Pioneer colonists, Intermediate colonists, Monoraphid, Araphid, size class 5 (large diatoms), Colonial, Centric, High profile, Low profile, LS5, HS4, LS3 and HS3 CEMFGs. In contrast, sites with Low densities of *T. granifera* were associated with Late colonist, Biraphid, smaller sized (size class 2 and 4), Mobile, Motile, HS2, HS5, MS1, MS2, MS3, MS4 and MS5 CEMFGs. Sites separated on the second axis (explaining 22.97%) due to water quality variables and *T. granifera* age groups. Olifants sites separated from the other sites below the axis due to the higher levels of conductivity (EC) as well as NO_3^- and SO_4^{2-} , while higher water temperature was associated with the Phongolo sites and higher pH and Cl^- with Limpopo sites. OLIF-2 and OLIF-3 had higher abundances of Juveniles and New-borns, while the Phongolo sites had more Mature adults.

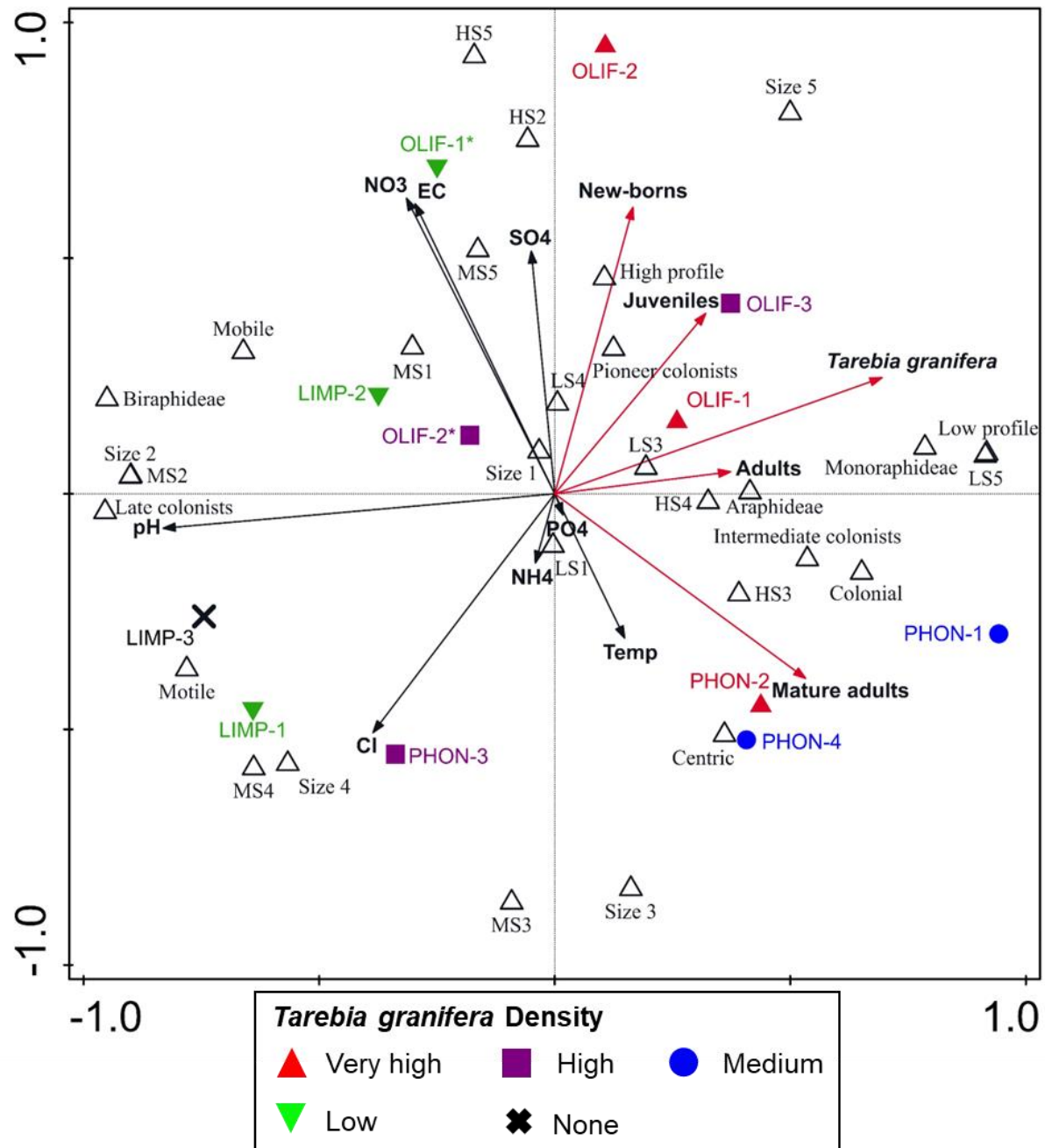


Figure 4.7: Principal Component Analysis (PCA) tri-plot of diatom functional groups (triangles) sampled at the selected sites from 2017 to 2021 with water quality variables (black arrows) as well as *Tarebia granifera* density and age groups (red arrows) plotted as supplementary variables. The tri-plot explains 57.83% of the total data variation of which 34.86% is displayed on the first axis and 22.97% on the second axis. Sites were grouped according to *T. granifera* density (None, Low, Medium, High and Very high) and age groups (Mature adults, Adults, Juveniles and New-borns). * Indicates Olifants sites sampled in 2021.

4.4 Discussion

Several studies have suggested that *T. granifera* invasions would have deleterious effects on aquatic communities and may alter food web dynamics within invaded habitats (Hillebrand *et al.*, 2002; Appleton *et al.*, 2009; Miranda *et al.*, 2010; Miranda *et al.*, 2011a; Miranda & Perissinotto, 2014; Hill *et al.*, 2015). In order to contribute to the knowledge of *T. granifera* invasions, the present study aimed to determine the ecological impacts of this invasive snail on benthic diatom community structures within invaded river ecosystems. Results indicated differences in diatom community structures between sites with higher densities of *T. granifera* and those with lower densities or no snails present. River sites with higher densities of *T. granifera* had overall lower diatom diversity, lower number of taxa as well as dominance of some diatom species and functional groups, while sites with lower densities of *T. granifera* had higher diversity, more diatom taxa, higher species richness and contributed to different diatom functional groups.

4.4.1 Diatom species community structures

From the present study, results indicated that *T. granifera* density had a substantial impact on benthic diatom community structures, sites with higher densities of *T. granifera* were dominated by several diatom species, while sites with lower densities of the snail had greater diatom diversity and evenness. Also, the single site that was dominated by the native *Melanoides tuberculata* had a higher number of diatom taxa and species richness compared to those of the Phongolo and Olifants (surveyed in 2020) rivers dominated by *T. granifera*. Several studies have indicated that high densities of grazers such as *T. granifera* can have a strong impact on diatom biomass and community structures (Hagerthey *et al.*, 2002; Sahan *et al.*, 2007; Armitage *et al.*, 2009). *Tarebia granifera* also have a higher carrying capacity than *M. tuberculata* (Miranda & Perissinotto, 2014; Raw *et al.*, 2015), and can have ingestion rates of up to 20 times that of other native snails which have strong impacts on microphytobenthic biomass dynamics within invaded aquatic ecosystems (Blanchard *et al.*, 2000; Miranda *et al.*, 2011a).

However, the site with no snails had the lowest number of taxa and species richness of all sites sampled during the 2021 survey. Begon *et al.* (1990) and McClatchie *et al.* (1982) found that diatom communities without grazers generally had lower diversity due to competition for nutrients between diatom species, leading to decreased number of diatom species and lower species richness. At high densities, snail excretions and bioturbation of sediments can play a role in fertilising diatom communities and sediments (López-Figueroa & Niell 1988; Widdicombe & Austen, 1999; Hagerthey *et al.*, 2002; Armitage *et al.*, 2009), changing the nutrient cycles of invaded aquatic ecosystems (Arnott & Vanni, 1996; Hall *et al.*, 2003; Carlsson *et al.*, 2004).

Changes in the availability of nutrients can affect food web dynamics, with unknown implications for ecosystem function (Moslemi *et al.*, 2012).

Sites sampled from the Olifants River were associated with higher levels of organic nutrients (PO_4^{3-} , NO_3^- and SO_4^{2-}), this may explain the high densities of *T. granifera* in the system as higher nutrients, especially nitrogen and phosphorus, increase primary production and thus food availability (Patrick, 1961; Lange-Bertalot, 1979; Vanni, 2002; Armitage *et al.*, 2009; Taylor & Cocquyt, 2019), likely facilitating the population growth of the invasive snails. However, during the present study, average NO_3^- concentrations did not differ much between the 2020 and 2021 surveys on the Olifants River, while both NH_4^+ and PO_4^{3-} concentrations were lower during the 2021 survey. Furthermore, the decrease in average *T. granifera* density and increase in the number of diatom taxa and species richness from 2020 to 2021 suggests that the 2021 survey likely had higher diatom taxa and species richness due to the decline in *T. granifera* density from 2020 to 2021. Especially since diatom community structures are often independently controlled by grazers or nutrients and these impacts often act separately (Hagerthey *et al.*, 2002; Elser *et al.*, 2005; Armitage *et al.*, 2009),

The dominance of some diatom species such as *Cocconeis*, *Gomphonema* and *Fragilaria* at sites with high densities of the invasive snail, is likely due to the grazing activity of *T. granifera*. Previous studies found that benthic diatom community structures were strongly impacted by grazing while the effects of nutrient enrichment were more variable and weaker (Hagerthey *et al.*, 2002; Armitage *et al.*, 2009). Nutrient enrichment increased the overall abundance of diatoms but did not alter diatom biomass (Armitage *et al.*, 2009). From an experimental study, diatom communities in the presence of *Hydrobia ulvae* (Pennant, 1777) snails had substantial similarity compared to those from the control groups without snails, and different treatments of temperature and nutrient enrichment had no significant effects on diatom species richness, evenness or diversity (Hagerthey *et al.*, 2002). However, *H. ulvae* consumed larger numbers of the benthic diatom biomass at higher temperatures and under high nutrient conditions, certain diatom species (*Amphora*, *Navicula* and *Nitzshia*) became more dominant (Hagerthey *et al.*, 2002).

4.4.2 Diatom functional group community structures

The use of diatom functional groups indicated that the links between such groups and environmental variables can be stronger than those with single species levels (Rimet & Bouchez, 2012). In terms of diatom colonisation, many events take place on substrates: diatom abundance and diversity increase by reproduction and immigration, while it decreases through grazing, mortality and emigration (Ács & Kiss, 1993). From the present study, sites with higher densities of *T. granifera* had higher abundances of Pioneer colonists than sites with lower densities. Thus,

the diatom communities from sites with higher densities were still in the earlier stage of colonization, as the abundance of Pioneer colonist species decreases during the final steps of the colonisation process (Stevenson, 1986; Ács & Kiss, 1993). These disturbances in the diatom communities from sites with higher densities of the invasive snail were most likely due to the grazing activity and high densities of *T. granifera* that has been reported to interfere with the natural trophic dynamics within invaded ecosystems (Pillay *et al.*, 2009). This suggests that the higher grazing pressure from *T. granifera* at sites with higher densities did not allow these diatom communities to become fully established and move into the final steps of colonisation. In contrast, sites with lower densities and the site with no *T. granifera* had higher abundances of Late colonists, while sites with higher densities had much lower abundances of Late colonists. As diatom species abundances of Late colonists increase during the final steps of the colonisation process (Stevenson, 1986; Ács & Kiss, 1993), sites with lower densities of *T. granifera* were representative of diatom communities in the final steps of the colonisation process likely due to the lower grazing pressure at these sites. The dominance of Intermediate colonist species across sites, regardless of *T. granifera* density, may be explained by the ability of such species to reproduce rapidly and these species usually have more stable abundances than the other two colonisation groups (Stevenson, 1986; Ács & Kiss, 1993).

As previously mentioned, *T. granifera* may select certain groups of diatoms over others (Miranda *et al.*, 2011a), from the present study it seems that *T. granifera* size may play a role in their diet preference. The higher abundance of larger diatom species at sites with higher densities of small snails might be as a result of smaller snails that graze more on smaller diatom species. However, this requires further investigation in future studies. As mentioned in Chapter 3, previous studies have indicated that juvenile snails can have higher impacts on food resources than larger adult snails (Boland *et al.*, 2008; Tamburi & Martín, 2008; Miranda *et al.*, 2011b).

Results from the present study further indicated that sites with higher densities of *T. granifera* had higher abundances of Monoraphid and Araphid diatom species, including *Cocconeis* sp., *Amphora* sp., *Gomphonema* sp., *Nitzschia* sp. and *Fragilaria* sp. that are large Intermediate and Pioneer colonist species. Monoraphids such as *Cocconeis* species persist on substrates through disturbances and have low migration ability (Luttenton & Rada, 1986; Stevenson & Peterson, 1989). In contrast, sites with lower densities of *T. granifera* had higher abundances of smaller Biraphid species such as *Craticula*, *Cymbella*, *Pleu. salinarum*, *Navicula* and *Tryblionella* species. These smaller Biraphid species can reproduce, migrate and colonise substrates faster than Monoraphid species (Stevenson & Peterson, 1989; Ács & Kiss, 1993). Biraphid species can also migrate rapidly due to their high motility and thus have a selective advantage as they can resist disturbances such as abiotic variables (water flow or nutrients) and have the ability to avoid

grazers (Peterson, 1987; Steinman *et al.*, 1987; Stevenson & Peterson, 1989; Passy, 2007; Rimet & Bouchez, 2011). After substrate disturbances such as grazing, fewer abundances of diatoms remain on the substrates and the ability of Biraphid species to rapidly colonise these substrates play a vital role in stabilizing/restoring these communities after disturbances (Ács & Kiss, 1993). The dominance of the adnate diatoms: *Cocconeis* sp. and *Amphora* sp. at sites with higher densities of *T. granifera* may also be explained by the stronger grazing pressure found on ribbon colony species compared to adnate diatom species (Julius & Theriot, 2010; Rimet & Bouchez, 2012).

Diatom life forms are useful morphological traits that have been indicated to have a strong connection to physical disturbances and the use of resources (Lange *et al.*, 2016; Marcel *et al.*, 2017; Lukács *et al.*, 2018). From the present study, the Mobile life form dominated across sites regardless of *T. granifera* density, while sites with higher densities of *T. granifera* had higher abundances of Colonial diatom life forms than sites with lower densities. The dominance of Mobile life forms and Motile guild species across sites during the present study may be explained by their ability to move to more favourable environments. Mobile species are able to move due to their raphe structure, while Motile species are made up of fast-moving species such as *Craticula*, *Navicula*, and *Nitzschia*, these species can resist environmental pressures such as grazing pressure and fast-flowing water (Passy, 2007; Rimet & Bouchez, 2011). These life forms also have the ability to rapidly move from nutrient-poor environments to more nutrient-rich environments (Johnson *et al.*, 1997). Colonial life forms were dominated by *Fragilaria* species and *Aul. granulata*, the ability of these species to form colonies can have an advantage as it may be a form of protection against grazers (Carotenuto & Lampert, 2004). Some diatoms e.g., *Fragilaria* species can also form a protective mucous substance and secrete polyunsaturated aldehydes (oxylipins), serving as a feeding deterrent and as a form of defence (Carotenuto & Lampert, 2004; Berthon *et al.*, 2011; Rimet & Bouchez, 2011), however, it is not clear if this mechanism could serve as a defence against *T. granifera* grazing. In contrast, sites with lower densities of the invasive snail had fewer abundances of these colony-forming species and thus, it is anticipated that the high abundances of colony-forming species at sites with higher densities of *T. granifera* could be linked to the grazing effects of the snails.

During the present study, the increase in the High profile guild and decrease in the Low profile guild from the surveys in 2020 to 2021 may be explained by the change in *T. granifera* grazing pressure between the two surveys. According to Passy (2007), the High profile guild comprises of large or colony-forming diatom species such as filamentous and branched diatoms that favour nutrient enrichment but is susceptible to grazing pressure and fast-flowing waters. The transition from Low profile to High profile diatom species also has been associated with a decrease in

grazing pressure and water flow disturbances (Steinman, 1996; Peterson, 1996). However, during the present study, water discharge was higher during the 2021 survey than in the 2020 survey on the Olifants River (refer to Chapter 2, Table 2.1), while *T. granifera* density decreased from 2020 to 2021, and as mentioned earlier, both NH_4^+ and PO_4^{3-} concentrations were lower during the 2021 survey compared to the 2020 survey. As such, the shift from Low profile to High profile diatom species during the 2021 survey is likely to be linked to the much lower grazing pressure of *T. granifera* during the 2021 survey.

From the present study, sites with higher densities of *T. granifera* had more than three times more Low profile guild species than sites with lower densities of the snail. Results from the present study also indicated that sites with lower densities of *T. granifera* had more evenly distributed CEMFGs with more of the smaller sized groups, while large-sized Low profile (LS5) CEMFGs had much higher abundances at sites with higher densities of *T. granifera*. Passy (2007) classified the Low profile guild as diatom species with resistance to water flow but with no tolerance for nutrient enrichment, this includes adnate, erect and prostrate diatom species. The higher abundances of large Low profile species at sites with high densities of *T. granifera* corresponds with the dominance of the large adnate diatom species (*Cocconeis* and *Amphora* species) at these sites. Diatom species of the Low profile guild are able to withstand resource limitation and environmental pressures (grazing and fast water flow), giving these species an advantage in ecosystems with high disturbance pressures and enabling them to become dominant (Passy, 2007). Previous studies found that Low profile species are adapted to high environmental disturbances regardless of nutrient availability and species such as *Cocconeis* can have high resistance to grazers (Biggs *et al.*, 1998; Passy, 2007). According to Biggs *et al.* (1998), high densities of snails can significantly alter diatom community structures towards higher abundances of diatom species with resistance to grazing pressures. Thus, during increased grazing pressure (or after such a disturbance) diatom communities are restricted to a certain group of taxa that are available to recolonise these disturbed environments (Biggs *et al.*, 1998).

Although, the effects of *T. granifera* on aquatic biodiversity have been difficult to quantify, in recent years, several studies have suggested that high densities of *T. granifera* often results in decreased benthic biodiversity and disrupted aquatic ecosystem function (Miranda *et al.*, 2011a, 2011b; Moslehi *et al.*, 2012; Raw *et al.*, 2013; Miranda & Personate, 2014). Large populations of *T. granifera* are known to significantly reduce algal standing stocks and exploit large numbers of microphytobenthos (photosynthetic diatoms, cyanobacteria, flagellates, and green algae), thus potentially causing top-down and cascading effects on ecosystem function (Miranda *et al.*, 2010, 2011a; Miranda & personate, 2014). At high densities, *T. granifera* may indirectly limit energy transfers within food webs and can result in decreased benthic macroinvertebrate biodiversity

(Miranda & Perissinotto, 2014; Hill *et al.*, 2015). Furthermore, as *T. granifera* disrupt trophic functioning, native species tend to shift their dietary preference (Miranda & Perissinotto, 2012). As discussed in Chapter 1, section 1.4, the native *A. ovata* started to feed less on microphytobenthos and more on branching algae after an increase in abundance of *T. granifera* (Miranda & Perissinotto, 2012). Thus, the impacts of *T. granifera* on available resources such as benthic diatoms may alter food web dynamics, with unknown implications on aquatic consumers and higher trophic levels (Moslemi *et al.*, 2012). Consequently, further research on the impacts of this invader on aquatic ecosystem function and biodiversity are urgently needed.

4.5 Conclusion

This study aimed to determine the impacts of *T. granifera* on benthic diatom community structures. The results from this study suggest that at high densities, *T. granifera* likely alters the community structures and diversity of benthic diatom communities within invaded habitats. At high densities, *T. granifera* grazing and biology structures benthic diatom communities, changing species abundance and species dominance patterns. Benthic diatom communities from river sites with high densities of *T. granifera* had an overall lower number of taxa, species diversity, species richness and disrupted functional groups. Declines in benthic diatom biodiversity can affect whole food webs as changes in one trophic level can lead to disturbances and cascading effects on aquatic ecosystem function, with unknown effects on higher trophic level species. However, it is difficult to draw concrete conclusions in terms of the effects *T. granifera* has on benthic diatom community structures from *in situ* ecosystems, due to the differences in environmental variables (temperature, nutrient availability, pollutants, water flow, water quality) as well as complex top-down and bottom-up trophic interactions between rivers and sites. Further field and experimental studies are needed to clarify these changes in diatom community compositions. Experimental studies such as microcosms might provide better insight as these closed systems can be easily monitored and the effects of environmental variables can be controlled.

Chapter 5: Impacts of the invasive snail, *Tarebia granifera*, on diatom community structures in the Phongolo River Floodplain, using a microcosm approach

5.1 Introduction

Determining the factors that drive the successful invasion of invasive species and understanding their ecological impacts within invaded ecosystems is one of the most important research questions in ecological studies (van Kleunen *et al.*, 2010; Xu *et al.*, 2016). Experimental studies such as microcosms can be useful assessments to indicate or predict the effects of certain species for instance invaders on the natural environment (OECD, 2006). These studies can be used to test and assess the impacts of invaders on the community structures and functions of aquatic ecosystems by simulating the natural environment (OECD, 2006; Wojdak, 2005; Wojdak & Mittelbach, 2007; Esparra-Escalera, 2018). Temporary wetlands such as floodplain pools can easily be mimicked by experimental studies such as outdoor microcosm studies (Jocque *et al.*, 2010; de Necker *et al.*, 2022).

Several studies have suggested that *Tarebia granifera* may have severe impacts on benthic biomass that could have implications on food availability for native aquatic species and result in decreased benthic biodiversity as well as disturbances in aquatic ecosystem function (March & Pringle, 2003; Miranda *et al.*, 2010, 2011a; Moslemi *et al.*, 2012; Raw *et al.*, 2013; Miranda & Perissinotto, 2014; Hill *et al.*, 2015). However, few have attempted to determine these impacts (Jones *et al.*, 2017; Weyl *et al.*, 2020) and there is a need to understand the impacts of this invader on the native biodiversity and aquatic community structures of invaded freshwater ecosystems within South Africa (Makherana *et al.*, 2022a). The *in situ* results from Chapter 4 indicated that high densities of *T. granifera* likely alters the community structures of benthic diatoms, however, these results need to be confirmed with experimental studies in a controlled environment in order to eliminate the effects of environmental variables.

Thus, this chapter aimed to use microcosms to evaluate the subsequent effects of *T. granifera* on benthic diatom community structures within a controlled environment. The hypothesis for this chapter was that high densities of *T. granifera* in an aquatic community would result in a decrease in benthic diatom diversity, disrupting benthic diatom community structures and functionality.

5.2 Materials and methods

5.2.1 Experimental design: microcosms

Due to one of the worst droughts (2015 to 2017) in South Africa's history (Baudoin *et al.*, 2017; Abbas *et al.*, 2019; de Necker *et al.*, 2020, 2021, 2022) and a lack of flood release from the Pongolapoort Dam (Dube *et al.*, 2015; Welicky *et al.*, 2017; de Necker *et al.*, 2020), almost all wetlands in the Phongolo River Floodplain dried up (de Necker *et al.*, 2020, 2021, 2022). These events resulted in a disconnection between the Phongolo River and its floodplain (de Necker *et al.*, 2020). Pre-drought surveys (done before 2015) by Smit *et al.* (2016) and Dube *et al.* (2017, 2019) confirmed the presence of *T. granifera* in both the Phongolo River and the floodplain. During the drought in 2017, no live *T. granifera* were found in any of the floodplain pans due to them drying out, however, estimates of pre-drought densities in these pans are between 3 000 to 30 000 individuals/m² (unpublished data).

The microcosm experiment took place next to the Phongolo River at the selected floodplain pan within the Ndumo Game Reserve in September 2017 (austral spring), see Figure 2.2i-j in Chapter 2 (section 2.1.1). Nine microcosms (1.2 x 1.2 x 0.6 m) were constructed from reinforced wire mesh and lined with a black plastic liner. The study area was isolated with shade netting to keep wild animals out (Figure 5.1). Fifty kilograms of dry floodplain sediment were collected and added to the microcosms to create a 3 cm thick sediment layer in each microcosm. Water from the Phongolo River (next to the floodplain) was pumped into a 1000 L water tank, microcosms were then filled with approximately 540 L of river water, ensuring each microcosm had a water depth of 40 cm. Aquatic communities present in the water and sediment were considered homogeneous as the water and sediment were sampled from the same areas. Two of the dominant plant species: the water primrose (*Ludwigia adscendens* (L.) H. Hara) and the swan plant (*Gomphocarpus physocarpus* E. Mey) growing in the selected pan were placed in the microcosms. These plants were added to simulate an environment as close as possible to that of the natural aquatic ecosystem in the selected area. Benthic diatoms were sampled in triplicate from each of the four sites on the Phongolo River as described in Chapter 4 (section 4.2.1). The samples were then mixed to create a homogenised sample that represent the diatom communities from the sampled sites. Each of the microcosm treatments were then seeded with equal portions (100ml) of the diatom mix and were left for a week to allow time for conditions to stabilise. The microcosms were divided into three treatment groups, each with three replicates, and seeded with either invasive, native or no snails. All *T. granifera* were measured in length (mm) before placing them into the microcosms and again after the experiment to ascertain if any growth took place during the experimental period. The first treatment of microcosms had no snails (Control), microcosms from the second treatment contained the invasive *T. granifera* snail (*Tarebia*) at a

density of 699/m² (representing the average density from the sites sampled in the Phongolo River) and the last treatment group were seeded at a density of 8/m² with the native *Melanoides tuberculata* snail (*Melanoides*). Only 24 *M. tuberculata* were collected in total from all sampling sites on the Phongolo River, subsequently, the three microcosms from the *Melanoides* treatment were seeded with eight snails each. After the snails were added, microcosms were left for five weeks before benthic diatoms were sampled. This is a sufficient period for diatom community succession and establishment (OECD, 2006; Stevenson *et al.*, 2010; Kock, 2020).



Figure 5.1: Microcosm layout and design with protective shade netting around the study area. Photo credit: Dr Lizaan de Necker.

5.2.2 Diatom sampling and identification

Prior to benthic diatom sampling, *in situ* water quality variables were measured and water samples (250ml) were collected for chemical nutrient analyses. Water quality measurements and nutrient analysis for each microcosm were done as described in Chapter 3 (section 3.2.1). Each microcosm was sampled separately in October 2017. Benthic diatom samples from each of the microcosms were sampled, prepared and identified to species level where possible, following the methods of Taylor *et al.* (2007a, 2007b) as described in Chapter 4 (section 4.2.1). Identified diatoms were then divided into several functional groups according to colonisation strategies (Stevenson, 1986; Ács & Kiss, 1993), size classes, life forms, ecological guilds (Rimet & Bouchez, 2011, 2012) and Combined Eco-Morphological Functional Groups (CEMFGs) (Viktória *et al.*, 2017), as described in Chapter 4 (section 4.2.1).

5.2.3 Statistical analysis

As a result of punctures to the plastic liner from vegetation overgrowth, three microcosms (one replicate from each treatment) were lost, and subsequent analyses were only applied to two replicates in each group. Statistical analysis was done as described in Chapter 4 (section 4.2.2). Primer version 7 software (Clarke & Gorley, 2006) was used to conduct univariate diversity indices, GraphPad Prism 7 was then used to create graphical representations of the diatom diversity indices. A Bray-Curtis similarity matrix was used to create a dendrogram of the diatom community data from the microcosm treatments. Treatments were grouped for analysis as *Tarebia* (invasive), Control (none), and *Melanoides* (native). These groupings were then tested for significance using the Analysis of Similarity (ANOSIM) procedure (Clarke & Gorley, 2006) using a preselected factor of treatment “snails”: none, native and invasive. Canoco version 5 (ter Braak & Šmilauer, 2012; Šmilauer & Lepš, 2014) was used to determine potential differences between the three microcosm treatments. An unconstrained Principal Component Analysis (PCA) was created using diatom community composition for each microcosm treatment. Water quality variables were excluded as supplementary variables due to their similarity across the different treatment groups. Student t-tests were used to test for significant differences ($p < 0.05$) between groups for selected variables (water quality variables and *T. granifera* growth/shell size).

5.3 Results

Measured *in situ* water quality and water nutrient parameters were similar within the different microcosm treatments (Appendix C1), and nitrites (NO_2) were below the detection limit for all microcosms. However, student t-tests revealed that microcosms with *T. granifera* had significantly higher conductivity (EC; $p = 0.034$), total dissolved solids (TDS; $p = 0.075$) and nitrates (NO_3 ; $p = 0.0195$). *Tarebia granifera* also showed significant ($p = 0.028$) growth in size during the five-week study period. The snails initially sampled from the river (used to seed the microcosms) had an average length of 9.33 mm (± 1 SD) and *T. granifera* collected from the two microcosms post-study had an average length of 11.35 mm (± 1 SD).

5.3.1 Microcosm diatom species community structures

A total of 35 benthic diatom species were identified from three microcosm treatment groups, 18 species were sampled from the Control treatments, 16 from the treatments with the native *M. tuberculata* and 18 from the treatments with the invasive *T. granifera*. Appendix C2 summarises the benthic diatom species abundances as well as different functional groups that were assigned to the taxa of the microcosms sampled. Diatoms that were unique to the microcosms without the invasive snail (Control and *Melanoides* treatments), included *Amphora veneta*, *Craticula*

molestiformis, *Eunotia flexuosa*, *Eunotia incisa*, several *Nitzschia* species, *Fragilaria ulna*, *Fragilaria ulna* var. *acus*, *Gomphonema angustatum*, *Gomphonema parvulum*, *Hantzschia amphioxys*, *Navicula radiosa*. Diatom species that were unique to the *Tarebia* microcosms, included *Bacillaria paradoxa*, *Craticula cuspidata*, *Craticula halophila*, *Cyclotella meneghiniana*, *Cyclotella ocellata*, *Fragilaria capucina*, *Gomphonema lagenula*, *Gomphonema pseudoagur* and *Nitzschia sigma*.

Microcosms containing the native *M. tuberculata* had the highest average number of diatom taxa (11) and individuals (149), with the lowest average number of taxa (10) occurring in microcosms containing the invasive *T. granifera* (Figure 5.2). The average number of individuals for the benthic diatom community was lowest in the Control (100) while the species richness was highest in Control (2.17) and lowest in the *Tarebia* microcosms (1.87). The average evenness and Shannon diversity were similar between all three microcosm treatments. However, it was highest in the *Melanoides* microcosms (0.93 and 2.2) and lowest in the *Tarebia* microcosms (0.88 and 1.99), respectively.

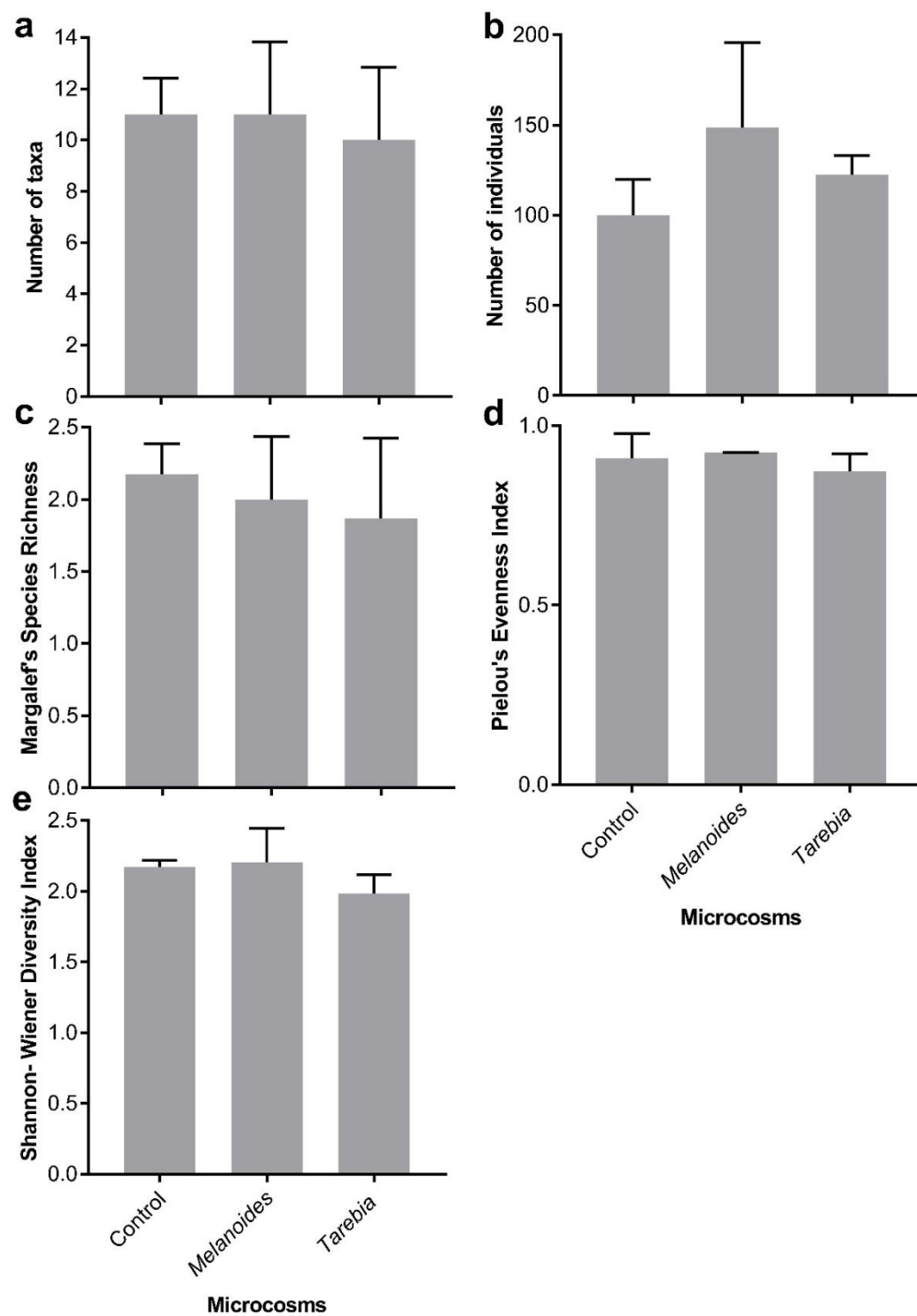


Figure 5.2: Mean values ($\pm$ SD) of combined benthic diatom diversity indices from microcosm treatments sampled in October 2017: a) the total number of taxa, b) number of individuals, c) Margalef's Species Richness, d) Pielou's Evenness Index, e) Shannon- Wiener Diversity Index.

The dendrogram comparing microcosm diatom community structures revealed that communities separated between 21.85 to 43.64% similarity, indicating substantial differences between microcosm treatments as well as diatom communities (Figure 5.3). Based on diatom species data, two groups formed on the dendrogram at 21.85% similarity, separating the diatom communities of the *Tarebia* 2 and Control 2 microcosms from the other treatment groups. Two further groups formed at 29.01% similarity, separating the *Tarebia* 1 treatment from the Control 1 and the two *Melanoides* treatments. The two *Melanoides* microcosm treatments shared the highest similarity (43.64%) in diatom community structure and separated from the Control 1 treatment at 35.04% similarity.

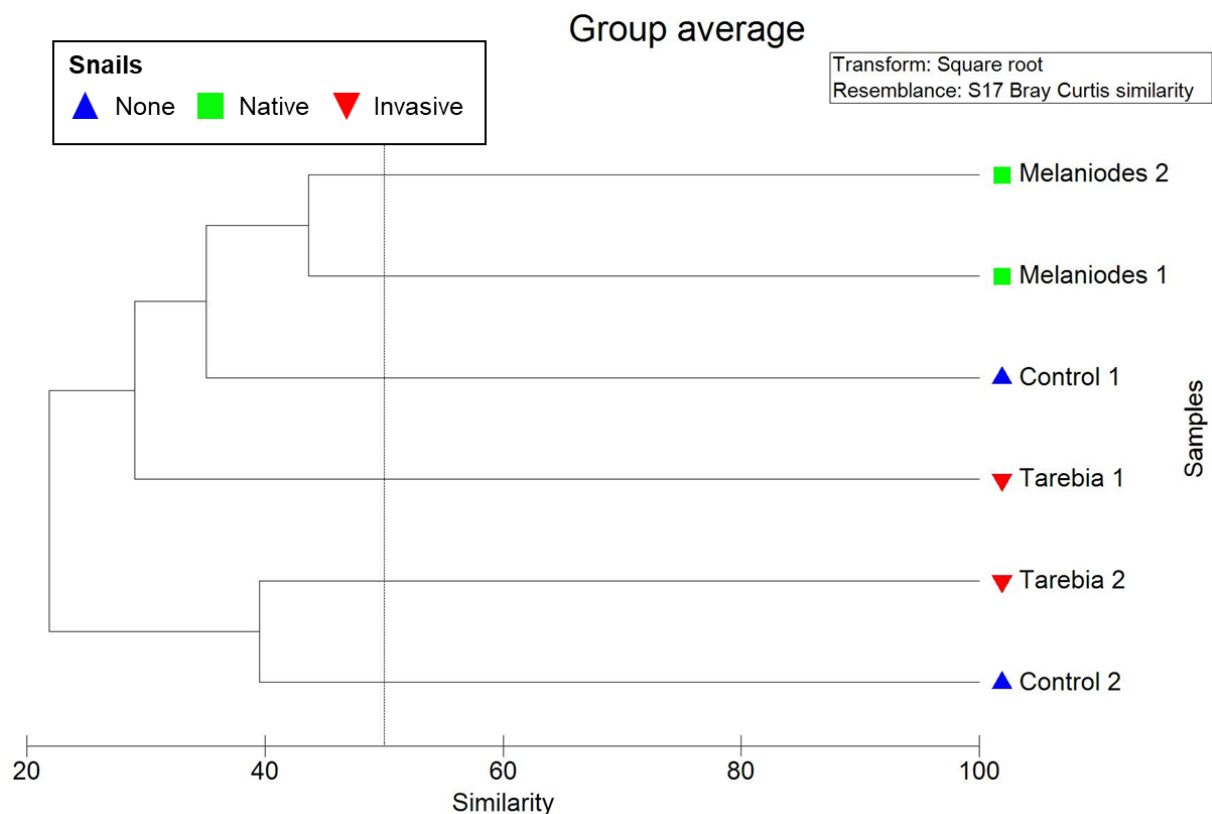


Figure 5.3: Dendrogram of benthic diatom species sampled from the three microcosm treatments in October 2017. Treatments had none (Control), invasive (*Tarebia granifera*), and native (*Melanoides tuberculata*) snails. The dotted line indicates 50% similarity. The resemblance between microcosms is based on Bray-Curtis dissimilarity coefficients calculated from diatom community structures.

The ANOSIM analysis based on the factor “snails” (none, native and invasive) indicated that diatom communities were substantially similar ($R = 0$), and groupings were not significant (40%). These similarities were as a result of the similarity between microcosm treatments without the invasive snail. Pairwise tests also revealed that diatom communities from microcosm treatments with none and the native ($R = 0.25$), none and invasive ($R = -0.5$), and native and invasive ($R = 0.25$) snails were all substantially similar and none of these groupings was significant ($> 5\%$).

However, the SIMPER pairwise analysis revealed major dissimilarities between diatom community structures based on the three different microcosm treatment groups (Appendix C4). Treatments with no snails and the native *M. tuberculata* were 67.20% dissimilar, however, they were the most similar in diatom community structure of all the treatment groups. The diatom species contributing the most to this dissimilarity were *Nitzschia recta* (6.82%), *Cra. molestiformis* (6.49%), *Nitzschia dissipata* (6.29%) and *Nitzschia linearis* (4.91%) that had higher abundances in microcosms with the native *M. tuberculata*, while *Fragilaria biceps* (6.28%), *Nitzschia etoshensis* (5.01%) and *Nitzschia dissipata* var. *media* (4.49%) had higher abundances in microcosms without snails. Microcosm treatments with no snails and the invasive *T. granifera* were 71.56% dissimilar in diatom community structure. The diatom species contributing the most to this dissimilarity were *Nit. etoshensis* (6.8%) and *Fra. ulna* var. *acus* (4.23%) that had higher abundances in microcosms without snails, while *Nit. linearis* (6.4%), *Gomphonema insigne* (6.32%), *Nit. dissipata* var. *media* (5.79%) and *Gom. lagenula* (4.35%) had higher abundances in microcosms with the invasive *T. granifera*. Treatments with the native (*Melanoides*) and invasive (*Tarebia*) snails had the highest dissimilarity (78.96%) in diatom species composition of all the microcosm treatments. The species that contributed the most to this high dissimilarity were *Nit. recta* (8.35%), *Cra. molestiformis* (5.46%), *Nit. linearis* (5.46%), *Nit. dissipata* (5.29%) and *Nit. etoshensis* (4.83%) that had higher abundances in the microcosms with the native *M. tuberculata*, while *Fra. biceps* (5.98%), *Gom. insigne* (5.3%) and *Nit. dissipata* var. *media* (4.94%) had higher abundances in microcosms with the invasive *T. granifera*.

The PCA of the 25 best fitting benthic diatom species sampled from the different microcosm treatments, explains 44.64% of the total data variation of which 29.41% is explained on the first axis and 15.23% explained on the second axis (Figure 5.4). The different microcosm treatments are grouped on the PCA based on differences in diatom species composition between treatments. Microcosms with the native *M. tuberculata* were the most similar in diatom community structure, these microcosms had higher abundances of *Cra. molestiformis*, *Gom. parvulum*, *Nit. recta* and *Nit. dissipata*. The following species were only present in microcosms with the invasive *T. granifera*: *Bac. Paradoxa*, *Fra. capucina*, *Gom. lagenula*, *Gom. pseudoagur* and *Nit. sigma*. Control microcosms had higher abundances of *Amphora* sp., *Fra. ulna* var. *acus*, *Fra. ulna* and *Gomphonema clavatum*.



Figure 5.4: Principal Component Analysis (PCA) of the 25 best fitting diatom species (circles) sampled from the different microcosm treatments. The PCA explains 44.64% of the total data variation of which 29.41% is displayed on the first axis and 15.23% on the second axis. Treatments were grouped for analysis according to snails: none (Control), invasive (*Tarebia granifera*), and native (*Melanoides tuberculata*).

5.3.2 Diatom functional group community structures

In terms of colonisation strategy, Intermediate colonists dominated in all microcosm treatments, with the highest abundance in *M. tuberculata* treatments (80.61%), while the Control (62.76%) and *T. granifera* (65.31%) treatments were similar (Figure 5.5a). Pioneer colonists had much higher abundances in the Control (37.24%) and *Tarebia* (27.76%) microcosms than in the *Melanoides* (5.78%) microcosms. Control microcosms did not have any Late colonists, while *Melanoides* microcosms (13.61%) had more Late colonists than *Tarebia* microcosms (6.94%). Biraphideae were the dominant taxonomic group sampled from all microcosm treatments, *Melanoides* microcosms (94.22%) had the highest abundance in this group followed by the *Tarebia* (70.61%) and Control (61.22%) microcosm treatments (Figure 5.5b). Monoraphid (1.62%) and Centric (3.27%) diatoms were only present in microcosms with the invasive *T. granifera*, while Raphidioideae (3.57%) was only found in Control microcosms. Both the microcosms without snails (35.20%) and those with *T. granifera* (24.49%) had the highest abundances of Araphid diatoms, while microcosms with *M. tuberculata* (5.78%) had the least.

Small-sized diatoms from size class 1 did not contribute to any of the diatom communities (Figure 5.5c). Microcosms without snails also did not have any size class 2 diatoms, while both the microcosms with *M. tuberculata* (6.46%) and *T. granifera* (5.71%) had similar abundances of size class 2 diatoms. Control (25.51%) and *Melanoides* (19.05%) microcosms both had higher numbers of medium-sized diatoms (Size 3) compared to *Tarebia* (9.39%) microcosms. Size class 4 diatoms dominated in *Melanoides* microcosms (53.74%), while size class 5 diatoms dominated in both the *Tarebia* (57.55%) and Control (42.86%) microcosms. The Mobile life form dominated in all microcosm treatment groups, the *Melanoides* treatments (94.22%) had the highest abundance of Mobile life forms followed by *Tarebia* (74.68%) and the Control (62.56%) treatments (Figure 5.5d). Pioneer life forms were absent from all microcosm treatments, while both the Control (37.44%) and *Tarebia* (25.32%) microcosms had higher abundances of Colonial life forms than the *Melanoides* treatments (5.78%).

The Motile ecological guild dominated in both *Melanoides* (84.10%) and Control (57.78%) microcosms, while *Tarebia* microcosms had slightly more of the High profile (49.79%) than the Motile (48.52%) guild (Figure 5.5e). Microcosms without snails did not have any of the Low profile guilds, while both *Melanoides* (1.77%) and *Tarebia* (1.69%) microcosms had similar low abundances of Low profile diatoms. Microcosms with the native *M. tuberculata* (14.13%) had lower abundances of High profile diatoms compared to the invasive *T. granifera* (49.78%) and Control (42.22%) microcosms. The HS1, LS1 to LS4 and MS1 CEMFGs did not contribute to any of the microcosm treatments (Figure 5.5f). The HS2 group were only present in *Tarebia* microcosms (5.91%), while small Motile diatoms were only present in *Melanoides* microcosms

(6.71%). Both HS3 and LS5 diatoms were only present in microcosms with the native (5.65% and 1.77%, respectively) and invasive snails (5.91 and 1.69%, respectively). Control and *Tarebia* microcosms were both dominated by HS5 diatoms, while *Melanoides* and Control microcosms had higher abundances of MS3 and MS4 groups than *Tarebia* microcosms.

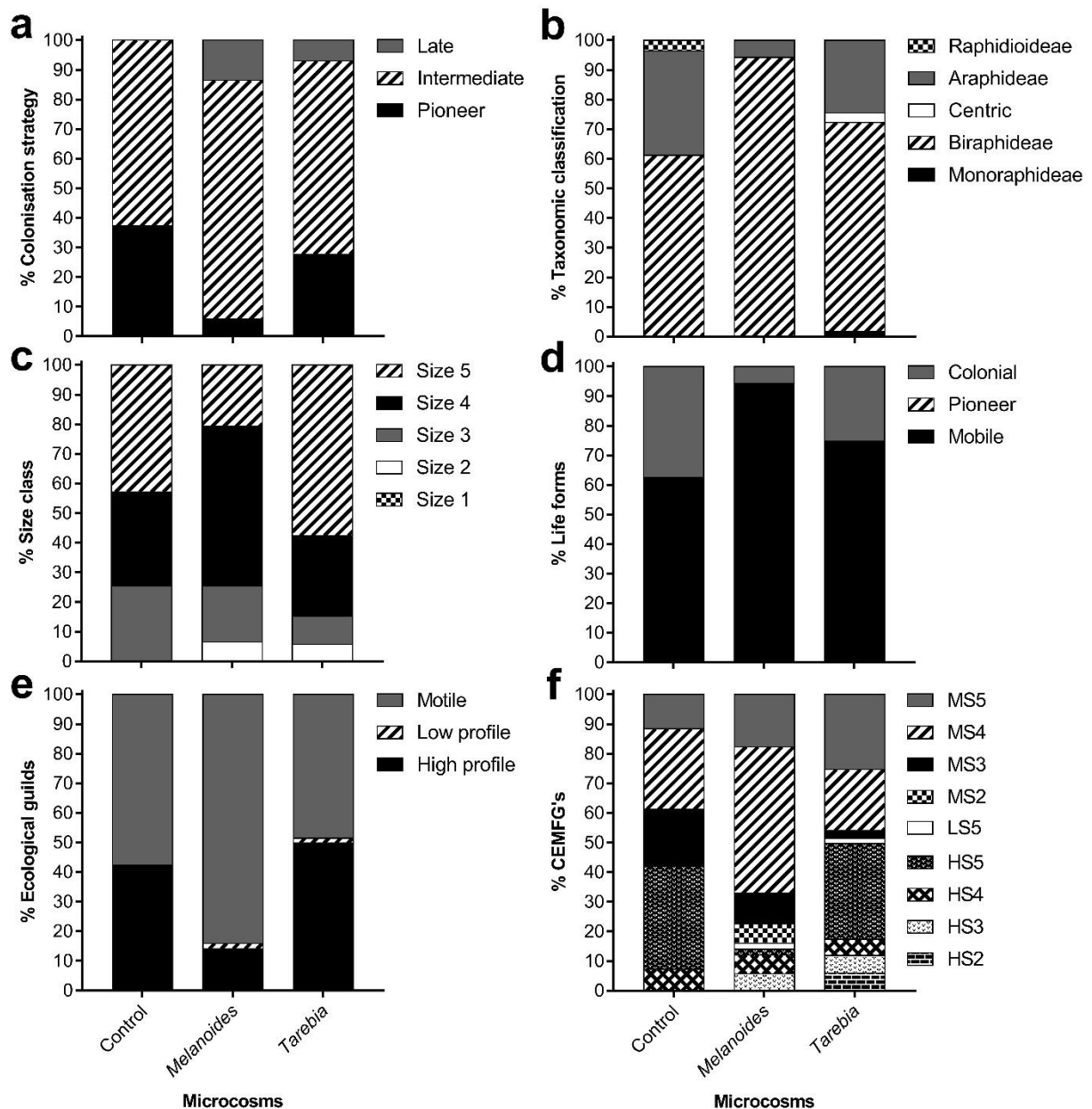


Figure 5.5: Percentage contribution (%) of diatom functional groups sampled from the three microcosm treatments in October 2017: a) colonisation strategy, b) taxonomic classification, c) size classes, d) life forms, e) ecological guilds, f) CEMFGs. Treatments had none (Control), invasive (*Tarebia granifera*), and native (*Melanoides tuberculata*) snails.

The dendrogram comparing benthic diatom functional group composition between the three microcosm treatments revealed that communities were very similar in terms of functional group composition (Figure 5.6). Communities separated at 70.86 to 87.44%, the microcosms with the native *M. tuberculata* were the most similar (87.44%) in diatom functional group composition and shared the highest similarity (82.54%) with the Control 2 diatom community.

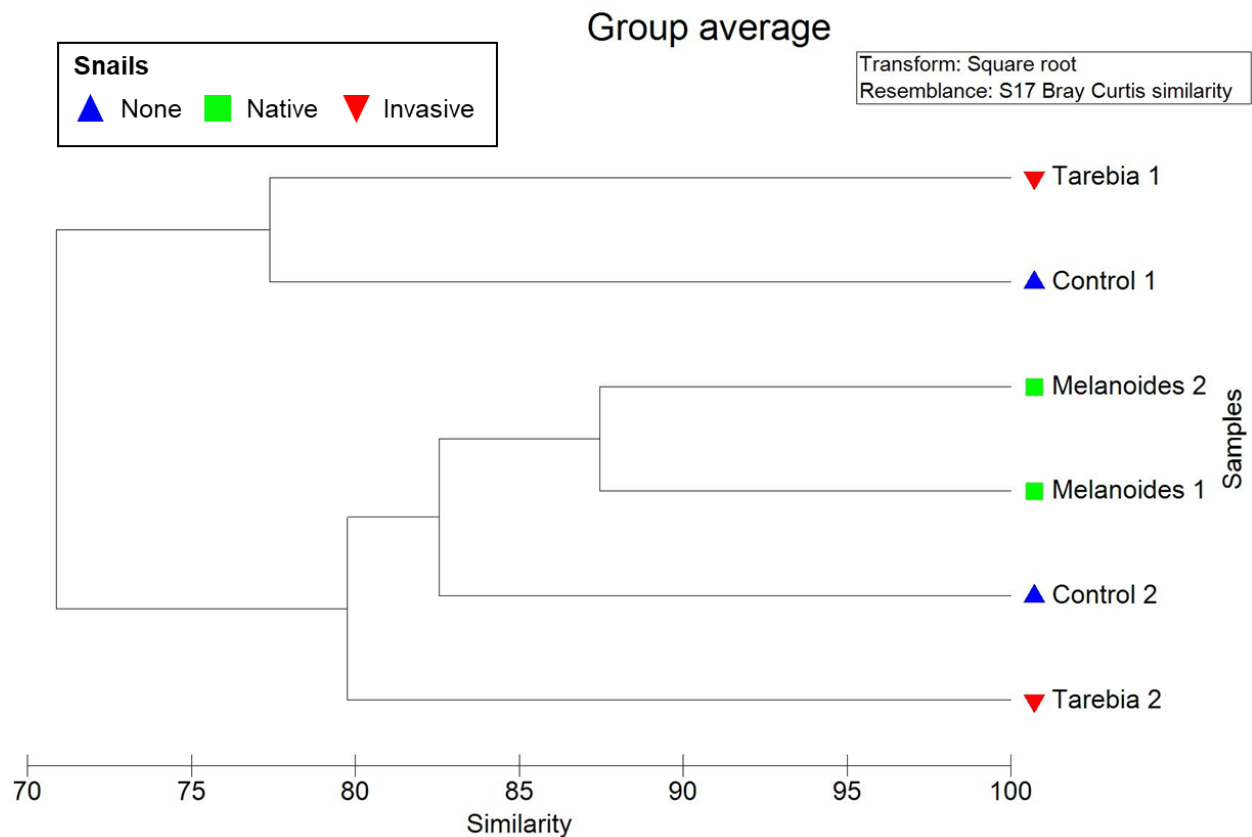


Figure 5.6: Dendrogram of diatom functional groups sampled from the three microcosm treatments in October 2017. Treatments had none (Control), invasive (*Tarebia granifera*), and native (*Melanoides tuberculata*) snails. The resemblance between microcosms is based on Bray-Curtis dissimilarity coefficients calculated from diatom community structures.

The ANOSIM analysis based on the factor “snails” (none, native and invasive) indicated that diatom communities from the different microcosm treatments were substantially similar ($R = 0.28$) in diatom functional group composition, and groupings were not significant (33.3%). However, ANOSIM pairwise tests revealed substantial differences in diatom functional group composition from the microcosms with no snails and those with the native *M. tuberculata* ($R = 0.5$), as well as microcosms with *M. tuberculata* and the invasive *T. granifera* ($R = 0.5$), although these groupings were not significant ($> 5\%$). Microcosms with no snails and those with the invasive *T. granifera* were substantially similar ($R = -0.5$) in functional group composition, but these groupings were also not significant ($> 5\%$).

The SIMPER pairwise analysis revealed low dissimilarities between diatom functional groups based on the three different microcosm treatment groups (Appendix C6). Microcosm treatments with no snails and the native *M. tuberculata* were 25.62% dissimilar in functional group composition. The diatom functional groups that contributed the most to this dissimilarity were HS5 (11.03%), Colonial (7.44%), Pioneer colonists (6.78%) and High profile (5.79%) diatoms that had higher abundances in microcosms without snails, while Late colonists (8.24%), MS2 (6.23%), Size 2 (6.13%) and HS3 (5.82%) diatoms were only present in microcosms with the native *M. tuberculata*. Microcosm treatments with no snails and the invasive *T. granifera* were 23.10% dissimilar in functional group composition. Diatom functional groups that had the highest contribution to this dissimilarity were MS5 (9.47%) and Late colonists (7.01%) that had higher abundances in treatments with the invasive *T. granifera*, while MS3 (8.66%), Pioneer colonists (6.96%), Size 3 (5.88%) diatoms were more abundant in treatments without snails. Microcosms with the native *M. tuberculata* and invasive *T. granifera* were the most dissimilar (26.10%) in diatom functional group composition of all the microcosm treatment groups. The functional groups that contributed the most to these differences were HS5 (10.55%), High profile (7.47%), Size 5 (6.64%), Pioneer colonists (5.97%) and Colonial (5.62%) diatoms that dominated in microcosms with the invasive *T. granifera*, while microcosms with the native *M. tuberculata* had higher abundances of MS5 (8.09%), MS4 (5.7%) and MS2 (5.94%) diatoms.

The PCA comparing differences in diatom functional groups between microcosm treatments explain a total data variation of 78.43% of which 54.84% is displayed on the first axis and 23.59% on the second axis (Figure 5.7). As with the dendrogram, the *Melanoides* microcosms were more similar in diatom functional group composition to one another and the Control 2 microcosms. *Melanoides* treatments were associated with Intermediate and Late colonists, Biraphid, Mobile and MS2 diatoms. While Control and *Tarebia* microcosms were associated with Size 5, Pioneer colonists, Araphid, Colonial, HS4 and HS5 diatoms.

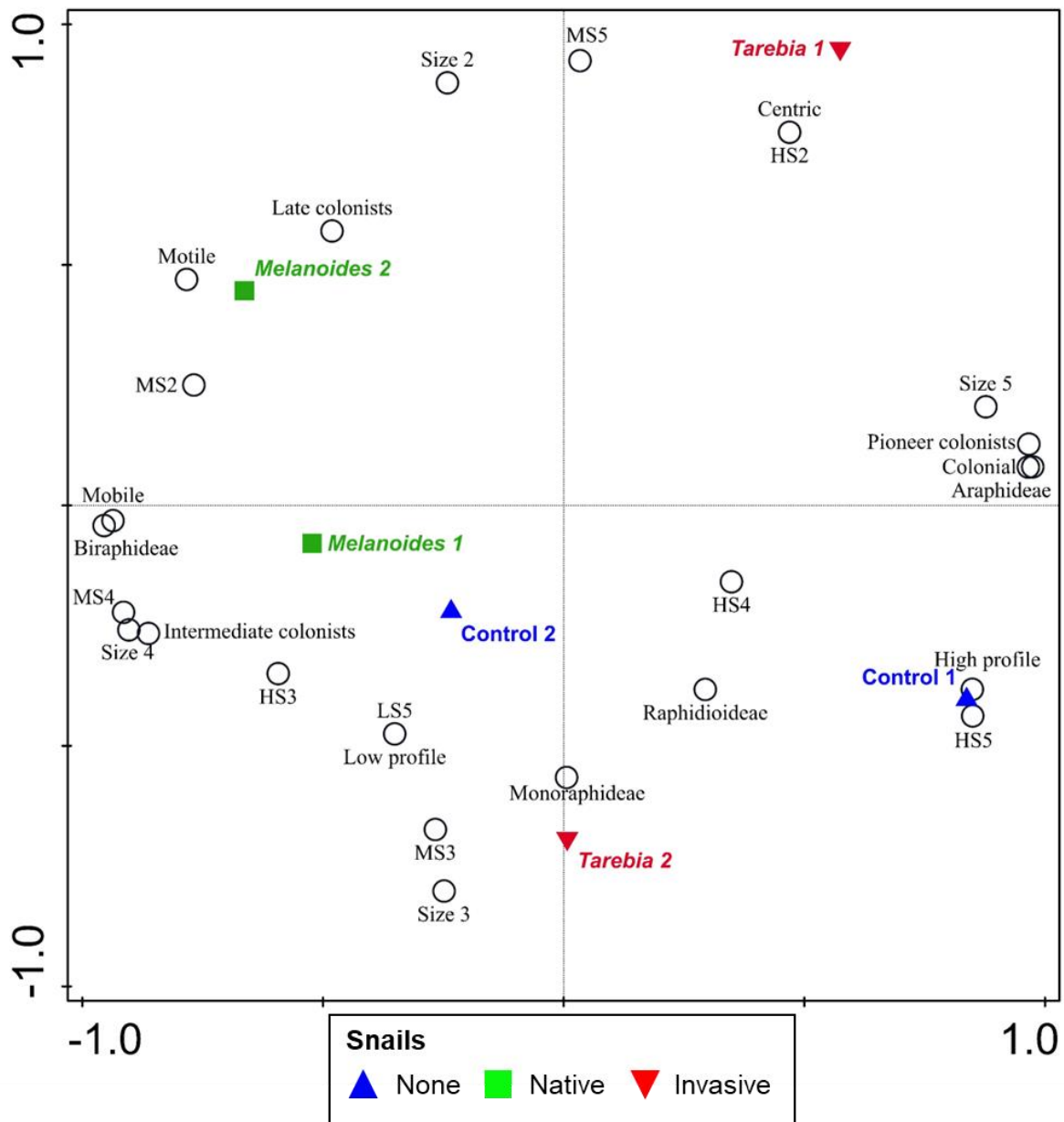


Figure 5.7: Principal Component Analysis (PCA) of diatom functional groups (circles) sampled from the different microcosm treatments. The PCA explains 78.43% of the total data variation of which 54.84% is displayed on the first axis and 23.59% on the second axis. Treatments were grouped for analysis according to snails: none (Control), invasive (*Tarebia granifera*), and native (*Melanoides tuberculata*).

5.4 Discussion

Introductions of invasive species to aquatic ecosystems have become a key ecological factor, as invading organisms increasingly alter the community structures of native species (Gurevitch & Padilla, 2004; Almeida *et al.*, 2018). The present study aimed to corroborate the findings of the *in situ* evaluation of the impacts of *T. granifera* on benthic diatoms (Chapter 4) with an experimental microcosm study. The results indicated only slight differences in water quality, but slightly greater differences in the diatom community structures from microcosm treatments containing the invasive *T. granifera* compared to the no snail and native *M. tuberculata* treatments. These differences included an average lower diatom diversity, fewer number of individuals as well as the dominance of some diatom species and functional groups. However, these results should be verified by larger scale follow-up studies, due to the low number of replicates in the present study.

5.4.1 Microcosm diatom species community structures

The increase in conductivity, TDS and nitrates found in microcosm treatments with the invasive snail, during the present study, may be as a result of *T. granifera* ecology and biology. At high densities, benthic snails are known to cause sediment and microphytobenthos resuspension (Jensen & Siegismund, 1980; Hunt *et al.*, 1987; Morrissey, 1988; Andersen, 2001; Kelaher *et al.*, 2003; Orvain *et al.*, 2004). Such disturbances in sediment deposits (bioturbation), lead to the resuspension of salts and nutrients that are trapped in closed aquatic ecosystems (Jolly *et al.*, 2008) such as floodplain pans. These nutrients dissolve back into the water column and become available to primary producers (López-Figueroa & Niell 1988; Widdicombe & Austen, 1999; Hagerthey *et al.*, 2002; Armitage *et al.*, 2009). Appleton *et al.* (2009) reported that *T. granifera* cause bioturbation as they are frequently moving in and through sediments. At high densities, this behaviour can lead to changes in the nutrient cycles of invaded aquatic ecosystems (Arnott & Vanni, 1996; Hall *et al.*, 2003; Carlsson *et al.*, 2004) and can affect food web dynamics, with unknown implications for ecosystem function (Moslemi *et al.*, 2012). For example, sediment disturbances caused at high densities of *T. granifera*, may contribute to sediment erodibility as well as affect the amount of sunlight available to photosynthetic microorganisms e.g., algae and diatoms which may influence the community structures of these organisms (Pillay *et al.*, 2007; Miranda *et al.*, 2011a). In addition, aquatic invertebrates may also be affected by such disturbances, especially in terms of their larval settlement and recruitment in sediments, as well as competition for food and habitat resources (Kelaher *et al.*, 2003) however, this needs further investigation.

From the present study, average diatom diversity was generally lower in microcosm treatments with *T. granifera* compared to those without the invasive snail. Furthermore, results indicated that diatom communities without the invasive snail (*T. granifera*) were more similar in community structure compared to the treatments with the invasive snail. Similar to the findings of the one site dominated by *M. tuberculata* in Chapter 4, diatom communities from microcosm treatments with *M. tuberculata* had a higher average number of diatom taxa, and species richness compared to the diatom communities from microcosms with *T. granifera*. Similar findings were also observed during an experimental study by Esparra-Escalera (2018) in Puerto Rico, mesocosm treatments with *T. granifera* and *M. tuberculata* (four replicates each) were seeded with 14 adult snails each. Treatments with *T. granifera* had reduced benthic biomass, diversity and disrupted periphyton community structures due to the dominance of cyanobacteria (such as *Chroococcus*, *Anabaena*, and *Microcystaceae* species) as a result of *T. granifera* grazing (Esparra-Escalera, 2018). In contrast, results found that treatment groups with *M. tuberculata* had higher benthic biomass and diversity compared to those of *T. granifera*, indicating that the two snail species have different effects on the abundance and diversity of benthic primary producers (Esparra-Escalera, 2018).

Under conditions of low per capita resource availability, *T. granifera* can outcompete other snails, and also have a higher carrying capacity than the native *M. tuberculata* (Miranda & Perissinotto, 2014; Raw *et al.*, 2015, 2016). High densities of the invasive snail have been reported to limit energy transfers within food webs (Miranda *et al.*, 2010), resulting in the aforementioned reduced species abundance and shifts in dominance patterns of benthic diatoms. As previously mentioned, *T. granifera* can have ingestion rates of up to 20 times that of other native snails which in return strongly impact the microphytobenthic biomass dynamics of invaded aquatic ecosystems (Blanchard *et al.*, 2000; Miranda *et al.*, 2011a). Such disturbances in diatom communities from microcosms with the invasive snail were most likely due to the high densities and grazing activity of *T. granifera* that has been reported to interfere with the natural trophic dynamics in ecosystems and cause biodiversity loss (Pillay *et al.*, 2009). These statements may explain the lower diatom species diversity and abundance from microcosms with *T. granifera* compared to those without the invasive snail.

During the present study, diatom community structures from the Control and *M. tuberculata* microcosms were typical of established communities, representative of standard uninvaded (in terms of *T. granifera*) aquatic ecosystems in South Africa, with the dominant diatoms belonging to *Nitzschia* species (Dalu *et al.*, 2014; Kock *et al.*, 2019). Indicating that the experimental period was of sufficient duration to establish stable diverse diatom communities. Similar to sites with higher densities of *T. granifera* in Chapter 4, diatom communities from the *T. granifera* microcosms had higher abundances of *Gomphonema*, *Fragilaria* and *Cocconeis* diatom species.

Changes in microphytobenthic community structures are an important consideration which is often not considered (Miranda *et al.*, 2011a). As mentioned earlier, *T. granifera* may select certain groups of algae and diatoms (which make up the microphytobenthos) over others, thus changing the biomass and community structures of these algal groups (Armitage *et al.*, 2009). Such changes in community structures include a decline in the abundance of some species and shifts in species dominance patterns (Hooper *et al.*, 2005). Both *Gomphonema* and *Fragilaria* species are indicators of eutrophic ecosystems (Taylor *et al.*, 2007b; Riato *et al.*, 2014; Kock *et al.*, 2019). The nutrient increase found in microcosm treatments with *T. granifera* can thus result in a more favourable environment and lead to an increase of eutrophic diatom (*Gomphonema* and *Fragilaria*) species. In contrast, microcosms with the native *M. tuberculata* were not dominated by these nutrient tolerant species. Thus, the changes in community structures from treatments with the invasive snail are likely linked to the high densities and grazing activity of *T. granifera*.

5.4.2 Microcosm diatom functional group community structures

Similar to the findings of Chapter 4, Intermediate colonists were the dominant colonisation strategy group regardless of the presence of *T. granifera*. However, both the Control and *Tarebia* microcosms had much higher abundances of Pioneer colonists, similar to the sites with higher densities of *T. granifera* in Chapter 4. While *Melanoides* microcosms had the highest abundances of Late colonists, similar to the sites with lower densities of *T. granifera* in Chapter 4.

In terms of diatom size classes, diatom communities from both the Control and *M. tuberculata* microcosms had higher abundances of medium-sized (Size class 3 and 4) diatoms, while diatom communities with *T. granifera* were dominated by large (Size class 5) diatoms. This was also similar to the findings of Chapter 4, where sites with higher densities of *T. granifera* were dominated by large diatoms while sites with lower densities had more medium-sized and more evenly distributed diatom size classes. Microcosms with the native snails had the highest abundance of Biraphid diatom species, while Control microcosms and those with the invasive snail had more Araphid species. The high abundances of Araphid diatoms in the Control and *Tarebia* microcosms are consistent with the high abundances of *Fragilaria* species found in these microcosms. The early phases in the colonization processes of diatom species are usually dominated by large-sized Araphid, Pioneer and Intermediate colonists, while the final stages in diatom colonisation are dominated by medium-sized Biraphid and Late colonist diatoms (Stevenson, 1986; Ács & Kiss, 1993). This suggests that the diatom communities with the native *M. tuberculata* were more representative of communities in the final steps of the colonisation process, while those communities without the native snail were representative of communities in the earlier stages of the colonisation process.

Diatom communities from the Control microcosms may be representative of communities in the earlier stages of the colonisation process due to competition for resources, as diatom communities without snails have been reported to compete for nutrients leading to a reduced number of diatoms (McClatchie *et al.*, 1982; Begon *et al.*, 1990). This also corresponds with the lower number of diatom individuals found in the microcosms without snails and the lower number of diatoms from the site without snails sampled on the Limpopo River (Chapter 4). However, the grazing activity and higher densities of *T. granifera* may never have allowed for the establishment of stable and diverse diatom communities in microcosms with the invasive snail. These microcosms had a reduced average number of taxa and individuals as well as lower species richness and diversity with disrupted diatom functional groups, similar to the sites with higher densities of *T. granifera* refer to Chapter 4 (section 4.4.1 and 4.4.2). After snail grazing, fewer diatoms remain on substrates, with higher abundances of diatom species with resistance to snail grazing pressures (Biggs *et al.*, 1998). Thus, diatom communities are restricted to a limited group of species that are available to recolonise these disturbed environments (Biggs *et al.*, 1998).

The Mobile life form was the dominant diatom life form group from all microcosm treatments during the present study, this group also dominated across sites regardless of *T. granifera* density in Chapter 4. Similar to the diatom communities from sites with higher densities (Chapter 4), the diatom communities from microcosms with *T. granifera* had more Colonial diatom life forms compared to those with the native snails. The high abundance of Colonial life forms in these microcosms was also due to the dominance of *Fragilaria* species in these microcosms, similar to the sites with higher densities of *T. granifera* (Chapter 4, section 4.4.2). As described in Chapter 4, *Fragilaria* species have defence strategies against grazing pressures (Carotenuto & Lampert, 2004; Berthon *et al.*, 2011; Rimet & Bouchez, 2011), which may explain the dominance of such species in the microcosms and sites with higher densities of the invasive snail. However, sites with lower densities of the invasive snail (Chapter 4) and microcosms with the native *M. tuberculata* snails had lower abundances of these colony-forming species. Thus, it is anticipated that the high abundances of these species in communities with higher densities of *T. granifera* could be linked to the higher densities of the invasive snails and differences between the grazing effects of *T. granifera* and *M. tuberculata* (Esparra-Escalera, 2018).

During the present study, the high abundance of the High profile guild in the microcosms with *T. granifera* was due to the dominance of *Gomphonema* and *Fragilaria* species. According to Passy (2007), the High profile guild comprises of large or colony-forming diatom species that favour nutrient enrichment such as the dominant *Gomphonema* and *Fragilaria* species found in the microcosms with *T. granifera*. As mentioned earlier (section 5.4.1), the nutrient increase found in microcosm treatments with *T. granifera* likely resulted in an increase and dominance of

Gomphonema and *Fragilaria* species. These shifts in diatom dominance patterns could likely be linked to the high densities and grazing effects of *T. granifera*. As mentioned in Chapter 4, snails can change nutrient cycles in aquatic ecosystems (Arnott & Vanni, 1996; Hall *et al.*, 2003; Carlsson *et al.*, 2004) and *T. granifera* have been reported to excrete more nitrogen at high densities (Moslemi *et al.*, 2012). These changes in nutrient availability affect the growth of primary producers (Vanni, 2002), thus diatom community structures and may alter food web dynamics with unknown implications on other native aquatic organisms (Moslemi *et al.*, 2012). The present study found similar results to Esparra-Escalera (2018), that indicated that *T. granifera* has negative effects on the community structures of benthic primary producers due to reduced periphyton biomass, diversity and disrupted community structures found in mesocosm experiments with *T. granifera*.

5.5 Conclusion

This study aimed to confirm the findings of the *in situ* study on the impacts of *T. granifera* on benthic diatom community structures, with the use of an experimental microcosm study. Even though a loss in replication of each of the treatments occurred the results from this study suggest that *T. granifera* is likely to alter the structure and diversity of native benthic diatom communities within invaded habitats. Microcosms without the invasive snail had diatom communities with higher species diversity, richness, and evenness, whereas microcosms with the invasive snail had lower diatom diversity as well as disrupted community structures and functionality. The results from this study revealed that *T. granifera* likely had a negative impact on benthic diatom community structures, corroborating the findings of Chapter 4. However, these findings should be verified by larger scale follow-up studies in the future, due to the low number of replicates in the present study. Furthermore, the invasive snail do not only pose a threat to native biodiversity but may also pose a health risk to human and animal health. Especially since *T. granifera* are known to be a host for a number of medically and veterinary important trematodes that are known to infect humans and animals within and outside of its native ranges. Therefore, there is a need to determine the role of *T. granifera* as hosts for trematode parasites within the invaded ecosystems of South Africa.

Chapter 6: *Tarebia granifera* as a host for digenean trematodes and its genetic diversity in the Limpopo and Phongolo River systems

6.1 Introduction

Thiaridae (Gill, 1871 (1823)) or trumpet snails such as *Tarebia granifera* and *Melanoides tuberculata* pose a severe threat to public health and are of veterinary importance as they transmit trematode parasites of fishes, birds, and mammals (Sodeman, 1991; Mitchell *et al.*, 2000; Veeravechsukij *et al.*, 2018a). Both *T. granifera* and *M. tuberculata* are known to be first intermediate hosts for diverse and prevalent species of trematodes that have been reported to affect the intestinal, respiratory, and hepatic systems of animals and humans (see Appendix D1 and D2). *Tarebia granifera* is well known as a first intermediate host for several trematode species that cause human diseases, especially in their native ranges (Veeravechsukij *et al.*, 2018a, 2018b). Classical hypotheses such as the “Spillover” and “Spillback” hypotheses are important to consider with regard to invasive host species (Chalkowski *et al.*, 2018). Spillover of parasites occurs when parasites from invasive host species are introduced into a new environment and subsequently infect native species, therefore posing a health risk to native species (Daszak *et al.*, 2000; Lymbery *et al.*, 2014; Iglesias *et al.*, 2015; Chalkowski *et al.*, 2018). Parasite spillback occurs when native parasites from a native host infect an invasive host, therefore increasing the risk to infect other native species (Hoberg *et al.*, 2002; Kelly *et al.*, 2009; Chalkowski *et al.*, 2018).

Intestinal trematodes such as *Haplorchis pumilio* (Looss, 1896), *Haplorchis taichui* (Nishigori, 1924) and *Centrocestus formosanus* (Nishigori, 1924) that infect both *T. granifera* and *M. tuberculata* have been reported to infect people in China, Thailand and Korea (Veeravechsukij *et al.*, 2018a). These trematodes use fish as second intermediate hosts and infect mammals and humans that consume infected fish (Krailas *et al.*, 2011, 2014, 2016). The oriental avian eye fluke *Philopthalmus gralii* (Mathis & Ledger, 1910) also uses both *T. granifera* and *M. tuberculata* as first intermediate hosts (Appendix D1 and D2). *Tarebia granifera* was also reported to serve as a host for exotic parasites in its invasive ranges in Jamaica (McKoy *et al.*, 2011) and Texas (Tolley-Jordan & Owen, 2008). *Tarebia granifera* from Texas (invasive) have been found to be infected with *P. gralii* (Tolley-Jordan & Owen, 2008), while ostriches (*Struthio camelus* Linnaeus, 1758) from farms in Zimbabwe have been found to be infected with *P. gralii* from *M. tuberculata* (Mukaratirwa *et al.*, 2005). Furthermore, studies on *M. tuberculata* as hosts for trematodes in South Africa have been neglected in recent years, since the last reports describing trematodes from *M. tuberculata* in South Africa date back to 1921 and 1938 (Faust, 1921; Porter, 1938).

It may be possible that *T. granifera* and *M. tuberculata* share the same species of trematodes in South Africa or that the invasive snail harbours exotic parasites that might be infecting fish, birds, mammals and potentially humans. Appleton *et al.* (2009) suggest that due to the rapid spread of the invasive *T. granifera* and resultant replacement of native *M. tuberculata*, such problems may become worse in the future. To date, no trematode species have been reported in *T. granifera* from South Africa (Miranda *et al.*, 2011a, 2011b; Weyl *et al.*, 2020). However, this situation could change, and it needs to be closely monitored in the future. Especially since *T. granifera* may serve as a host for invasive parasites that could be brought in via migratory birds, or eventually serve as a host for native parasite taxa that spillover from native hosts. Consequently, studies on thiarid species and their parasitic diversity in South Africa will contribute to a better understanding of the biodiversity, biology and evolutionary relationships of the snail hosts and their trematodes.

Several distinct morphs of *T. granifera* exist worldwide, with a high degree of variation in shell morphology (Veeravechsukij *et al.*, 2018b). Appleton *et al.* (2009) reported two distinct morphological variants of *T. granifera* in KwaZulu-Natal, South Africa. One with a pale brown whorl and a dark spire, while the entire shell of the other is dark brown to almost black (Appleton *et al.*, 2009). Veeravechsukij *et al.* (2018b) found two genetically different clades within the *T. granifera* population of Thailand. Due to these morphological variations found in *T. granifera*, molecular phylogenetic and phylogeographical analyses are needed to distinguish between the different morphotypes (Veeravechsukij *et al.*, 2018b), this will provide an objective and comparable method for identification (Schols, 2019). According to Veeravechsukij *et al.* (2018b) and Yin *et al.* (2022), molecular studies on the phylogenetic diversity of *T. granifera* are lacking. Furthermore, the genetic diversity of *T. granifera* in South Africa remains unexplored. Since both parasites and snail hosts can show a high degree of cryptic diversity, and since many host-parasite associations are highly species-specific, it is important to accurately identify the lineages of *T. granifera* that could serve as potential trematode hosts in invasive regions. Therefore, studies on the genetic diversity of *T. granifera* populations from South Africa are needed and phylogeographical analysis may shed some light on where this invader came from, and which potential trematodes may be able to infect *T. granifera* in South Africa.

The aims of this chapter were thus: 1) to investigate the role of the invasive thiarid (*T. granifera*) versus the native thiarid (*M. tuberculata*) as hosts for digenean trematodes through the natural emergence of cercariae from the snails, with snail dissections and molecular analyses, and 2) to determine the genetic diversity of *T. granifera* from the selected sites on the Limpopo River system and Phongolo River with the use of molecular and phylogenetic analyses. The hypotheses for this chapter were: 1) *T. granifera* acts as a first intermediate host for medically and ecologically important trematodes within the invaded ecosystems sampled, which may spillover to native

species, and 2) due to the absence of males and the parthenogenic reproduction of *T. granifera*, the snails will have a low genetic diversity in South Africa.

6.2 Materials and methods

6.2.1 Snail sampling and screening for trematode species

Snails (*T. granifera* and *M. tuberculata*) were collected from sites on the Limpopo River system from April to May 2021 as indicated in Chapter 3. *Tarebia granifera* were also sampled from the PHON-4 site (Chapter 3) on the Phongolo River during a survey in November 2021. Snails were sampled by handpicking from the vegetation and stones for a period of 30 minutes during which time all snails found were collected following methods as described by Nguyen *et al.* (2021). Larger individuals were targeted during sampling, as these snails have a higher chance of being infected with trematodes (Giannelli *et al.*, 2016). Snails were identified to species level as described in Chapter 3 (section 3.2.1). Species were separated into one-litre jars (approximately ten snails per jar) filled with filtered river water (Tolley-Jordan & Owen, 2008) and fed with shredded lettuce (Schuster *et al.*, 2014). To ensure snails had adequate oxygen, water (filtered river water from the site) was changed once a day and snails were kept at room temperature away from direct light to reduce stress and overheating (Schols, 2019). The parasite infection status of snails was then evaluated with shedding experiments in the field. Individual snails were placed in separate cups filled with filtered river water and exposed to a light source for one hour (mimicking sunlight) for three consecutive days to induce the release of trematode cercariae (Schwelm *et al.*, 2020). Each cup was screened four times (every 20 minutes) for the presence of cercariae using a Nikon stereomicroscope (Nikon Instruments, Tokyo, Japan). After the shedding experiments, individual snails were fixed in labelled Eppendorf tubes with 96% ethanol. Samples were then preserved by freezing them (-4 °C) on-site in a mobile freezer until analyses could be conducted in the laboratory at the North-West University (NWU), Potchefstroom. Prior to fixation, the length of each snail was measured with a digital calliper (Kroeplin B2R20S) with an accuracy of 0.1 mm. All snail samples were collected and processed with the necessary permits (Permit No. ZA/LP/107276) from the Limpopo Department of Economic Development, Environment and Tourism, Ezemvelo KZN Wildlife (Permit No. OP 864/2021) and ethical clearance from the North-West University AnimCare Animal Research Ethics Committee (Ethics No. NWU-01539-20-A9).

6.2.2 Molecular identification of snails and trematodes

For the purposes of molecular identification and to determine prepatent trematode infections, the ten largest snails per species per site were selected for analysis. As previously mentioned, the larger individuals were selected as older snails have a higher chance of being infected with trematodes (Giannelli *et al.*, 2016). Snail shells were carefully crushed with pliers and the entire snail was removed from its shell, whereafter a small piece of each snail's foot tissue and the hepatopancreas were removed using sterile forceps and scalpel blades (Schuster *et al.*, 2014; Schwelm *et al.*, 2020; Nguyen *et al.*, 2021). To prevent contamination between samples, equipment was sterilised with bleach after each individual dissection (Schols, 2019). The snail foot tissue and hepatopancreas samples were subsequently preserved in separate, labelled Eppendorf tubes with 96% molecular grade ethanol and stored in a freezer (-20 °C) until further analyses.

A piece of each of the snail foot tissue samples was cut with sterile scalpel blades and placed in separate sterile Eppendorf tubes for analysis. The hepatopancreas samples were divided in two with sterile scalpel blades, one half of each sample was crushed and placed in separate sterile Eppendorf tubes for further analysis. After removing as much of the ethanol from the tubes as possible, the remaining ethanol from each sample was evaporated in a heat block at 56 °C. The total genomic DNA was extracted from both the snail foot tissue and hepatopancreas samples with the use of the PCR Biosystems Rapid DNA Extraction Kit (PCR Biosystems available from Analytical Solutions, Randburg, South Africa). The manufacturers protocol was followed: 20 µl lysis buffer, 10 µl proteinase K buffer and 70 µl molecular grade water was added to each sample. Samples were then incubated at 75 °C for 5 minutes (mixing the samples twice by vortex in the 5 minutes); then at 95 °C for 10 minutes. After incubation, samples were diluted with 150 µl molecular grade water instead of 900 µl as recommended, in order to obtain DNA at a higher concentration. The samples were then centrifuged at 15 596 RCF for one minute. Finally, 150 µl of the supernatant from each sample was transferred to sterile tubes and frozen (-20 °C) to prevent DNA degradation.

The DNA samples were amplified by polymerase chain reaction (PCR) protocols for target gene fragments using the relevant primers. These primers were chosen based on preliminary identification of snail species or trematodes as recommended by previous studies (Folmer *et al.*, 1994; Tkach *et al.*, 2001, 2003; Veeravechskij *et al.*, 2018a, 2018b; Schols, 2019; Lopes *et al.*, 2020; Vermaak, 2021). All primers used for DNA amplification during this study are summarised in Table 6.1. Three genetic markers were targeted for DNA amplification (one for snails and two for trematodes). For the amplification of the cytochrome c oxidase mitochondrial gene complex (cox1), LCO1490 forward and HCO2198 reverse primers (Folmer *et al.*, 1994) were used. The

cox1 genetic marker was used to identify snails due to its high evolutionary speed (Hebert *et al.*, 2003) and it is well represented in sequences found on Genbank (NCBI, <https://www.ncbi.nlm.nih.gov/>) (Schols, 2019). The remaining two genetic markers were used to identify trematode infections in the snails. The Internal Transcribed Spacer 2 region (ITS2) was amplified with the 3S forward primer (Bowles *et al.*, 1993) and ITS2.2 reverse primer (Cribb *et al.*, 1998). For amplification of the 28S ribosomal DNA, the D12 forward (Tkach *et al.*, 2001) and 1500R reverse (Tkach *et al.*, 2003) primers were used. Additionally, two internal primers: 300F (forward) and ECD2 (reverse) were used to sequence the 28S genetic marker. The PCR thermocycling protocols for each genetic marker and primer sets are summarised in Figure 6.1.

For each targeted genetic marker, a PCR master mix containing the appropriate PCR reagents was prepared. By using a master mix, the amount of pipetting is reduced, the risk of contamination is minimized, possible mixing errors are prevented, and it also saves time (West & Sawyer, 2006). The *cox1* PCR master mix was made up of 8 µl double-distilled water (ddH₂O), 1.25 µl of both the forward (LCO1490) and reverse (HCO2198) primers, and 12.5 µl Kapa Hifi Hot Start Taq DNA polymerase per sample. The master mix (23 µl) and DNA template (2 µl) were added in the Eppendorf tubes and the samples were amplified in a SimpliAmp ThermoCycler machine following PCR conditions in Figure 6.1a. For the ITS2 genetic marker, the PCR master mix consisted of 7 µl ddH₂O, 1.25 µl of both the forward (3S) and reverse (ITS2.2) primers and 12.5 µl Dream Taq DNA polymerase per sample. The master mix (22 µl) and DNA (3 µl) were added in the Eppendorf tubes and the samples were amplified following PCR conditions in Figure 6.1b. Finally, the 28S PCR master mix contained 8 µl ddH₂O, 1.25 µl of both the forward (D12) and reverse (1500R) primers, and 12.5 µl Dream Taq DNA polymerase per sample. The master mix (23 µl) and DNA (2 µl) were added in the Eppendorf tubes and the samples were amplified under PCR conditions illustrated in Figure 6.1c. DNA amplification success was determined by agarose gel (1%) electrophoreses; the gel was stained with SafeView Classic DNA dye. Successful amplicons were sent to Inqaba Biotechnical Industries Pty. Ltd. in Pretoria, South Africa for purification and sequence generation.

Table 6.1: Primers used for DNA amplification and sequencing during this study. The table was adapted from Vermaak (2021).

Genetic marker	Primer	Sequence	Reference
cox1	LCO1490	5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'	Folmer <i>et al.</i> (1994)
	HCO2198	5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'	Folmer <i>et al.</i> (1994)
ITS2	3S	5'-GGT ACC GGT GGA TCA CGT GGC TAG TG-3'	Bowles <i>et al.</i> (1993)
	ITS2.2	5'-CCT GGT TAG TTT CTT TTC CTC CGC-3'	Cribb <i>et al.</i> (1998)
28S	Dig12	5'-AAG CAT ATC ACT AAG CGG-3'	Tkach <i>et al.</i> (2001)
	1500R	5'-GCT ATC CTG AGG GAA ACT TCG-3'	Tkach <i>et al.</i> (2003)
(internal)	ECD2	5'-CTT GGT CCG TGT TTC AAG ACG GG-3'	Tkach <i>et al.</i> (2003)
(internal)	300F	5'-CAA GTA CCG TGA GGG AAA GTT G-3'	Tkach <i>et al.</i> (2003)

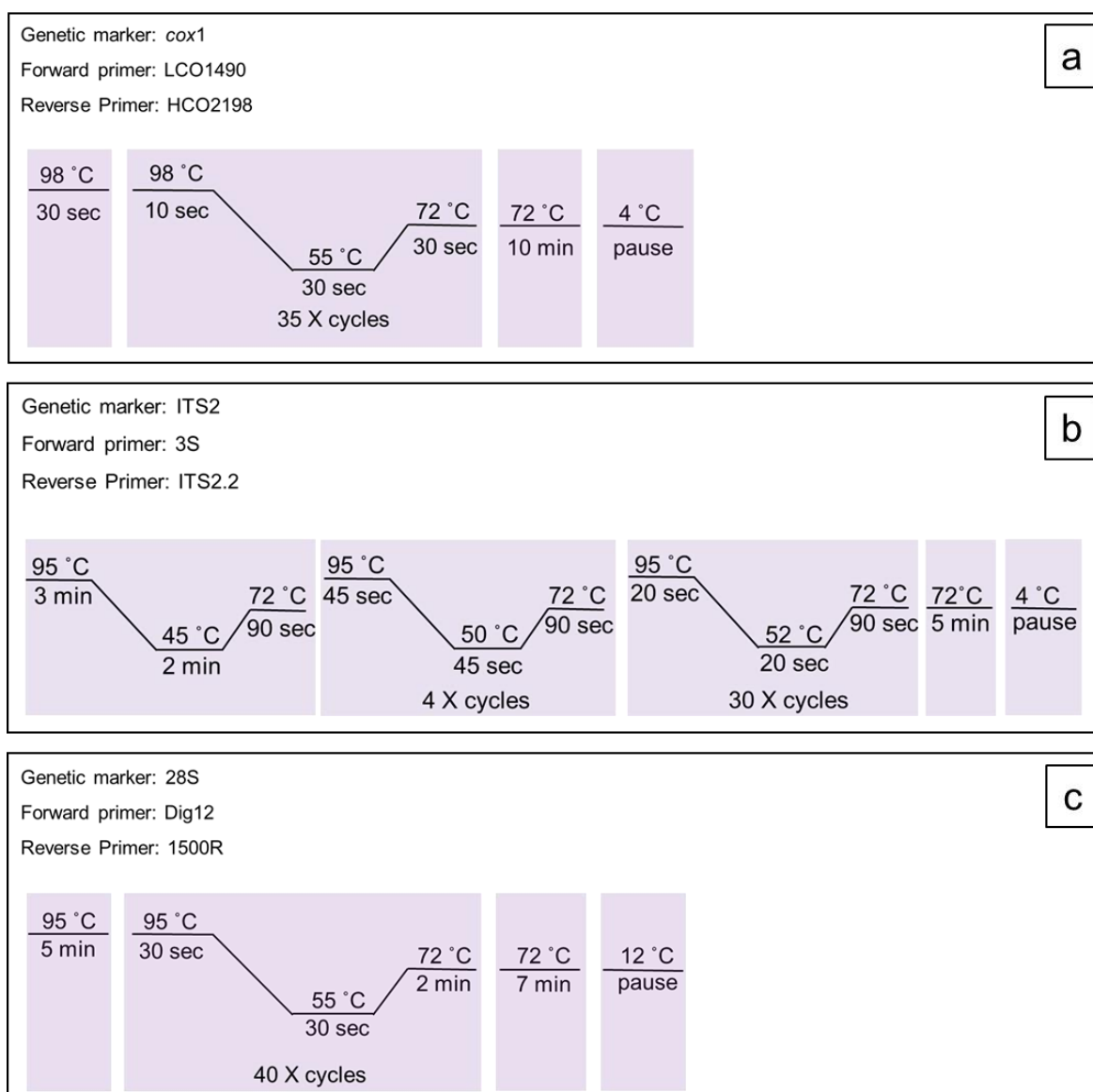


Figure 6.1: The PCR protocols used to amplify DNA of the three selected genetic markers: a) cox1, b) ITS2, and c) 28S. Protocols were adapted from Vermaak (2021).

Geneious v.11 bioinformatics software (Biomatters, Auckland, New Zealand) was used for sequence assembly, trimming and alignment of the obtained sequences. Trematode (ITS2 and 28S) and *T. granifera* (*cox1*) sequences obtained in the present study were compared with relevant sequences of trematode and snail species available in GenBank (NCBI, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) by using the Basic Local Alignment Search Tool (BLAST), to determine genetic similarities for species identification.

Tarebia granifera sequences (*cox1*) from the present study were compared with one another as well as the available *T. granifera* sequences in GenBank to determine genetic similarities between *T. granifera* from different regions (sites and countries). The alignments were constructed using MUSCLE (Edgar, 2004) implemented in Geneious v.11 under default parameter values. The *cox1* alignment included eight sequences from the present study (one *T. granifera* sequence per site) and ten sequences from Genbank, with *M. tuberculata* selected as an outgroup based on the phylogenetic analysis by Von Gersdorff Sørensen *et al.* (2005) and Harding *et al.* (2019). Only one *T. granifera* sequence per site was used due to all *T. granifera* sequences from the present study being 100% identical (see Results section). The sequences from the present study and GenBank sequences used for the phylogenetic analysis during this study are given in Appendix D3.

Prior to further analysis, the best fitting model for each alignment was determined with the use of jModelTest v.2.1.2 software (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) according to the Akaike information criterion (AIC). The HKY + I model was the best fitting model for the *T. granifera cox1* dataset. Bayesian inference was performed by MrBayes v.3.2.6 software (Ronquist *et al.*, 2012) and maximum likelihood analysis was carried out by RaxML-HPC v.8 on XSEDE (Stamatakis, 2014) run on CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010) (available at <https://www.phylo.org/>). The Markov chain Monte Carlo (MCMC) algorithm was used for the Bayesian inference analysis, the chains were run for 10 000 000 generations and sampled trees every 1 000 generations. The “burn-in” parameter was set to disregard the first 25% of the sampled trees, using only the remaining 75% of sampled trees to create the consensus trees (Hoogendoorn *et al.*, 2019). For all alignments, the nodal support (from the maximum likelihood analysis) was validated by applying 100 bootstrap pseudoreplicates. The phylogenetic tree was then visualised by using FigTree v.1.4 software (Rambaut, 2012). Pairwise genetic distance (p-distance) matrices as percentage similarity (%) and the number of base pair (bp) differences between the sequences used for phylogenetic analysis (Appendix D4) were calculated in Geneious v.11 software.

6.3 Results

6.3.1 *Tarebia granifera* and *M. tuberculata* as hosts for parasitic trematodes

In total 294 *T. granifera* and 62 *M. tuberculata* were collected from the Limpopo River system and 44 *T. granifera* were collected from the Phongolo River for the examination of trematode infections. All sampled *T. granifera* and *M. tuberculata* were mature adults (> 10.39 and > 12.64 mm, respectively) according to the size classes used in Chapter 3 (section 3.2.2). All dissected *T. granifera* were found to be females, with embryos in their brood pouches. Table 6.2 indicates the number of snails collected and screened for cercariae with the average snail length sampled from the selected sites. Neither of the two snail species shed cercaria during any of the shedding experiments. However, the molecular study investigating snail trematode infections resulted in the detection of two species of trematodes in *M. tuberculata* from the LIMP-1 site on the Limpopo River, while all *T. granifera* from all sites were found to be uninfected.

Table 6.2: Number of snail specimens shell length (average $\pm$ SD) and length range (mm) sampled at the selected sites.

Site Code	Sampling Date	River	Snail species	Number of snails sampled	Average snail length (mm) $\pm$ SD	Snail length range (mm)
PHON-4	16/11/2021	Phongolo	<i>Tarebia granifera</i>	44	12.17 $\pm$ 2.61	18 – 7.5
LIMP-1	22/04/2021	Limpopo	<i>Melanoides tuberculata</i>	62	18.49 $\pm$ 5.02	11.5 – 29.3
LIMP-2	25/04/2021	Limpopo	<i>Tarebia granifera</i>	67	16.76 $\pm$ 2.3	12.2 – 22.6
MOGA	26/04/2021	Mogalakwena	<i>Tarebia granifera</i>	24	14.53 $\pm$ 2.49	11 – 19.4
LUVU	29/04/2021	Luvuvhu	<i>Tarebia granifera</i>	24	15.01 $\pm$ 1.76	12.5 – 19.7
OLIF-1	05/05/2021	Olifants	<i>Tarebia granifera</i>	30	15.57 $\pm$ 2.28	12.1 – 21
OLIF-2	03/05/2021	Olifants	<i>Tarebia granifera</i>	33	16.95 $\pm$ 2.5	10.9 – 22.3
G-LETA	06/05/2021	Great Letaba	<i>Tarebia granifera</i>	78	15.47 $\pm$ 1.86	11.8 – 20
LETA	04/05/2021	Letaba	<i>Tarebia granifera</i>	38	16.28 $\pm$ 2.11	12.5 – 24.1

The sequences (ITS2 and 28S) from the two trematode species found in *M. tuberculata* were compared to the available sequences in GenBank to identify the trematode species. Sequences from the present study revealed that five of the ten *M. tuberculata* were infected with a *Haplorchis* sp. and one with a *Centrocestus* sp. A comparison of the ITS2 sequences (626 bp) obtained during the present study from the *Haplorchis* sp., with the best (highest % similarity) available sequences in GenBank revealed that the *Haplorchis* sp. from the present study is 98.36% similar to *H. pumilio* from Peru (Lopes *et al.*, 2020; GenBank accession No. MT829204). The genetic divergence between the *Haplorchis* sp. from South Africa (present study) and *H. pumilio* from Peru was 1.64% (6 bp). Comparison of the 28S sequences (1 182 bp) obtained during the present study from the *Haplorchis* sp., with the best available sequences in GenBank revealed that the *Haplorchis* sp. from the present study is 98.89% similar to *H. pumilio* from Thailand (Thaenkham *et al.*, 2010; GenBank accession No. HM004173), Vietnam (Le *et al.*, 2017; GenBank accession No. KX815125 and KY369156), Peru (Pulido-Murillo *et al.*, 2018; GenBank accession No. MG738252), and Brazil (Lopes *et al.*, 2020; GenBank accession No. MT840091). The genetic divergence between the *Haplorchis* sp. from South Africa (present study) and *H. pumilio* from the aforementioned countries was 1.11% (13 bp).

A comparison of the ITS2 sequences (626 bp) obtained during the present study for *Centrocestus* sp., with the best available sequences in GenBank revealed that the *Centrocestus* sp. from the present study is 98.45% similar to a *Centrocestus* sp. from Israel (Dzikowski *et al.*, 2004; GenBank accession No. AY245699). The genetic divergence between the *Centrocestus* sp. from South Africa (present study) and *Centrocestus* sp. from Israel was 1.55% (7 bp). A comparison of the 28S sequences (1 182 bp) obtained during the present study from the *Centrocestus* sp., with the best available sequences in GenBank revealed that the *Centrocestus* sp. from the present study is 96.86% similar to *C. formosanus* from Peru (Pulido-Murillo *et al.*, 2018; GenBank accession No. MG738251), and Vietnam (Le *et al.*, 2017; GenBank accession No. KY351633 and KY369154). The genetic divergence between the *Centrocestus* sp. from South Africa (present study) and *Centrocestus* sp. from the aforementioned countries was 3.14% (37 bp).

6.3.2 The genetic diversity of *T. granifera*

All *T. granifera* *cox1* sequences from the present study were found to be genetically identical (100% similar), thus only one *T. granifera* sequence per site was used for the phylogenetic analysis. Within the phylogenetic tree based on the *cox1* dataset (Figure 6.2), *T. granifera* sequences (657 bp) from the present study formed a highly supported clade with sequences of *T. granifera* from China (GenBank accession No. MZ662113) and Thailand (GenBank accession No. MK000375). There was no intraspecific divergence observed between the aforementioned *cox1* sequences of *T. granifera* within this clade, thus the sequences from the present study were

100% identical to those from China and Thailand. The overall intraspecific variation between the *T. granifera* sequences from the present study and those of the other reference sequences used for analyses was between 1.8 to 3.6% (11-21 bp), with *T. granifera* from the USA (GenBank accession No. MT671805) and Thailand (GenBank accession No. MK000294) indicating the lowest divergence, while *T. granifera* from Singapore (GenBank accession No. AY958762) indicated the highest intraspecific divergence. For the *cox1* sequences, the interspecific divergence between *T. granifera* from the present study was lowest for *Tarebia lineata* (Gray, 1828) from Malaysia (GenBank accession No. MW591171) with 14.2% (93 bp), while the highest interspecific sequence divergence was observed for *Tarebia lineata* from India (GenBank accession No. KY511306) with 16.1% (106 bp).

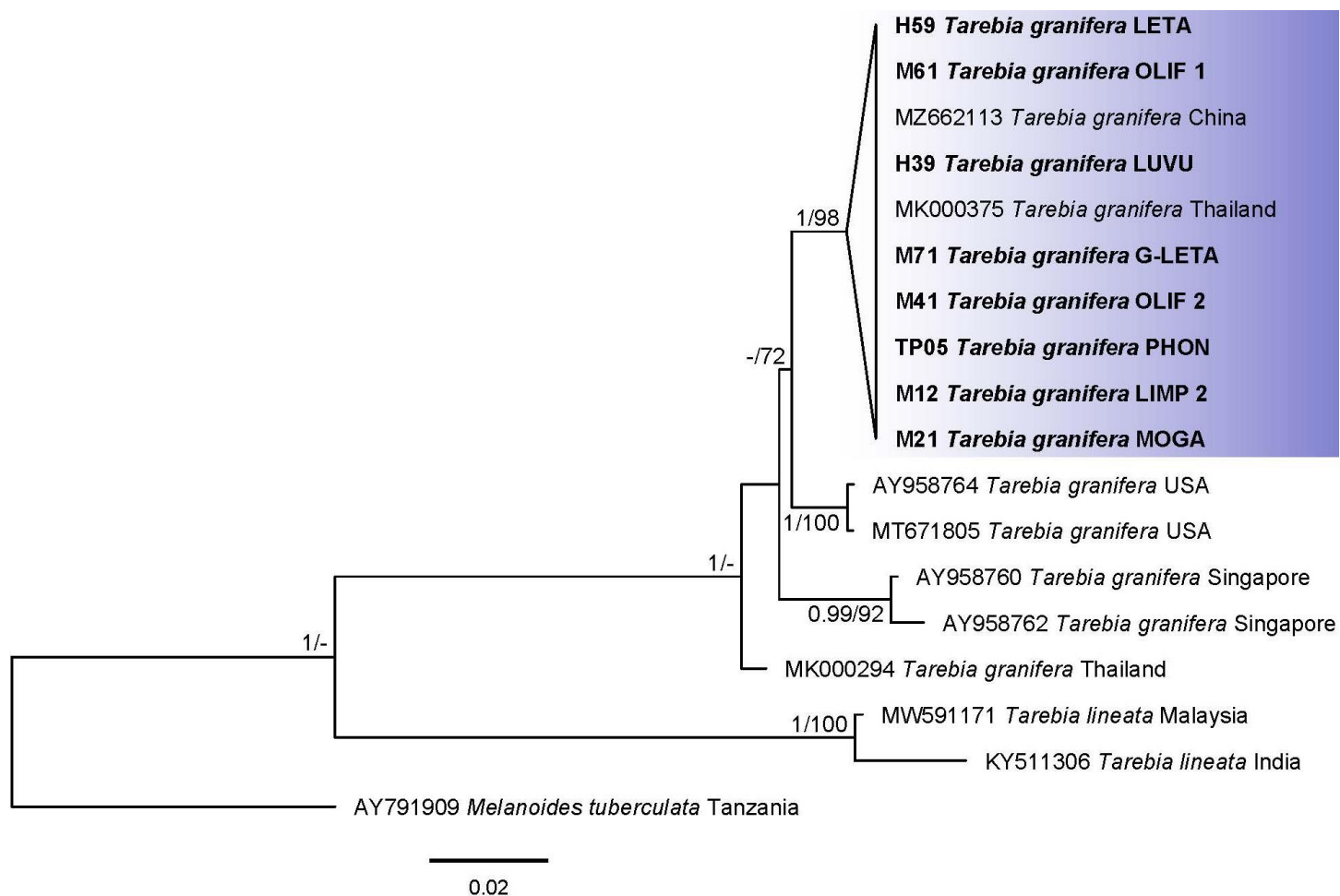


Figure 6.2: Bayesian inference (BI) phylogenetic tree for representatives of Thiariidae based on eight *cox1* sequences obtained from *T. granifera* during the present study and ten sequences obtained from GenBank (see Appendix D3). Support values are indicated on the nodes as BI/ML (maximum likelihood). Values lower than 0.9 (BI) and 70 (ML) were excluded. The expected number of substitutions per site are indicated by the scale bar. Sequences from the present study are highlighted in bold with the sample number followed by the snail species and site code. Reference sequences include the GenBank accession number followed by the snail species and country of origin.

6.4 Discussion

Freshwater snails and their parasitic trematodes are understudied worldwide (Adema *et al.*, 2012; Veeravechskij *et al.*, 2018a). Therefore, there is an urgent need for research on these snail hosts and the trematodes they may harbour (Veeravechskij *et al.*, 2018a). Furthermore, studies on the genetics and phylogeny of *T. granifera* may contribute to the control and prevention of disease-causing trematodes that use this snail as a host (Yin *et al.*, 2022). This study aimed to investigate the role of the invasive thiarid (*T. granifera*) versus the native thiarid (*M. tuberculata*) as hosts for digenean trematodes within the Limpopo River system and Phongolo River, as well as to investigate the genetic diversity of *T. granifera* in the selected study area. The invasive thiarid was found to be free from trematode infection, while the native thiarid was found to be infected with two species of trematodes within the study area. Furthermore, all *T. granifera* samples from the selected sites were found to be genetically identical, suggesting that all *T. granifera* in South Africa come from few or even a single invasion event.

6.4.1 *Tarebia granifera* and *M. tuberculata* as hosts for parasitic trematodes

During the present study, all *T. granifera* samples were found to be females and free from trematode infection. Miranda *et al.* (2011b) reported similar findings in *T. granifera* from KwaZulu-Natal. The invasive snails (*T. granifera*) may be free from trematodes, as exotic parasites may not have migrated with the host or the parasites could have been unable to survive within the newly invaded environment due to a lack of suitable intermediate or definitive hosts (Keane & Crawley, 2002; Torchin *et al.*, 2003; Genner *et al.*, 2008). The invasive snail may also be immune to native trematode infections due to evolutionary adaptations (Ebert, 1994, 1998; Prenter *et al.*, 2004; Fromme & Dybdahl, 2006). As trematodes co-evolved with their hosts, invasive species may have resistance or immunity against native parasites (Genner *et al.*, 2008). Therefore, trematodes that are native to South Africa have co-evolved with and specialised to infect native snails and *T. granifera* might be an unsuitable host for these native trematodes.

A review study by Preston *et al.* (2022) found that invasive snails had up to 80% lower parasite diversity within invaded habitats compared to their native ranges. Invasive species may have an advantage over native species when they have no parasites and/or no predators within invaded habitats (Genner *et al.*, 2008). Trematodes play a vital role in host snail populations as they can affect changes in survivorship, behaviour, morphology and cause reduced fecundity and growth of their snail hosts (Sorensen & Minchella, 2001; Lafferty & Kuris, 2009; McKoy *et al.*, 2011). Trematodes can act as parasitic castrators and cause reduced population growth within snail populations (Genner *et al.*, 2008; Weyl *et al.*, 2020), thus *T. granifera* that are free from trematode infections in South Africa likely have an advantage in terms of reproduction and contribute to rapid

population expansion (Weyl *et al.*, 2020). This correlates with the population densities of *T. granifera* in South Africa with several studies reporting densities of several thousand individuals/m² (Appleton *et al.*, 2009; Miranda *et al.*, 2011b; Miranda & Perissinotto, 2014; Jones *et al.*, 2017). Such advantages can have considerable effects on the population dynamics and competitive interactions among invaders and native species (Byers & Goldwasser, 2001; Genner *et al.*, 2008).

For the first time in South Africa, this study found the native thiarid, *M. tuberculata*, to be infected with *Haplorchis* sp. and *Centrocestus* sp. trematodes. This indicates that the trematode diversity from thiarid snails in South Africa is higher than previously reported in the literature. Trematodes from the family Heterophidae which includes *Haplorchis* sp. and *Centrocestus* sp. have been reported to cause gigantism and castrations in infected *M. tuberculata* with considerable effects on snail population growth and abundances (Genner *et al.*, 2008). Although *M. tuberculata* is native to South Africa (Connolly 1939; Brown 1994; Appleton, 2002), invasive morphs or hybrids from Asia may be present (Pointier *et al.*, 2003; Genner *et al.*, 2004; Raw *et al.*, 2015). Therefore, it may be possible that invasive morphs *M. tuberculata* may host parasites that are exotic in South Africa. However, the genetic diversity of *M. tuberculata* will first need confirmation with molecular analysis (Appleton & Miranda, 2015). Although no *T. granifera* have been found with trematode infections in South Africa, this may become a problem in the future as *T. granifera* are known to be the first intermediate hosts for several species of zoonotic trematodes within and outside of their native ranges (see Appendix D1 and D2), this includes species of *Haplorchis* and *Centrocestus* that were found to infect the native thiarid during the present study. Considering the parasite “Spillback” hypothesis (Hoberg *et al.*, 2002; Kelly *et al.*, 2009; Chalkowski *et al.*, 2018), it may be possible that parasites from native thiarid snails could infect (spillback into) *T. granifera* populations in the future. Especially since several studies have reported native parasite spillback into invasive/introduced species (Hemmingsen *et al.*, 2005; Krakau *et al.*, 2006; McAllister & Bursey, 2016; Chalkowski *et al.*, 2018). This could have severe risks for wildlife and humans in the future due to the rapid spread and high densities reached by *T. granifera* (Appleton *et al.*, 2009).

Veeravechsukij *et al.* (2018b) found that Thai *T. granifera* with trematode infections had lower reproductive output than uninfected individuals. According to the “Enemy Release” hypothesis, the absence of enemies such as parasites and predators within invaded ecosystems contribute to the invasion success of introduced species (Chalkowski *et al.*, 2018). Thus, having no trematode parasites that influence their population growth in South Africa and the several other characteristics of *T. granifera* (see Chapter 1 section 1.2.) enable them to quickly become the dominant species and displace native thiarids (such as *M. tuberculata*) within invaded

ecosystems. Several studies have indicated that *T. granifera* displaces native snail species, especially *M. tuberculata* in South Africa (de Kock & Wolmarans, 2008; Appleton *et al.*, 2009; Miranda *et al.*, 2011a; Miranda & Perissinotto, 2012, 2014; Jones *et al.*, 2017). Although the mechanisms responsible for this displacement are not fully understood (Raw *et al.*, 2013, 2015), the present study highlights the importance of trematode infections that play a role in the competitive interactions among invasive and native freshwater snail species as well as snail population control agents (i.e., as castrators).

Despite the numerous negative effects and threats that *T. granifera* poses to our native biota, as outlined throughout the present study, the snail may prevent the spread of the lung fluke disease (paragonimiasis) in KwaZulu-Natal (Appleton, 2014). *Melanoides tuberculata* is known to be the first intermediate host for *Paragonimus westermani* (Kerbert, 1878) in Asia (Nakagawa, 1917; Davis *et al.*, 1994; Blair *et al.*, 1999). According to Appleton (2014), the first intermediate host for the trematodes causing the disease must be *M. tuberculata* or *Tomichia natalensis* (Connolly, 1939). Chaniotis *et al.* (1980a) previously suggested that *T. granifera* was also an intermediate host for *P. westermani*, however, this theory was disproved by Michelson (1992). Thus, as *T. granifera* displaces *M. tuberculata* and *T. natalensis*, this may bring an end to the spread of paragonimiasis in KwaZulu-Natal (Appleton, 2014). *Tarebia granifera* has also been used in several countries as biological control agents for snail hosts of *Schistosoma* trematodes leading to the successful eradication of bilharzia in some of these regions (Prentice, 1983; Perez *et al.*, 1991; Sodeman, 1991; Pointier & Jourdane, 2000; Appleton *et al.*, 2009; Pointier *et al.*, 2010; Hewitt & Willingham, 2019). As mentioned in Chapter 3, *T. granifera* seems to have replaced *Bulinus tropicus* and *Bulinus forskalii* from the sites sampled on the Limpopo River system. Both these snail species are known to be hosts for *Schistosoma* trematodes (Tumwebaze *et al.*, 2019). It is therefore also possible that the invasive *T. granifera* acts as an unanticipated biological control agent for bilharzia within the invaded ecosystems of South Africa, however, further research would have to be conducted on the topic.

6.4.2 The genetic diversity of *T. granifera* in South Africa

Genetic analysis from the present study indicated that *T. granifera* from the Limpopo River system and the Phongolo River were all genetically identical based on mitochondrial DNA (*cox1* gene). This was expected, as invasive snails usually have considerably lower genetic diversity within their invasive ranges compared to their native ranges (Facon *et al.*, 2003; Hanson *et al.*, 2013; Ebbs *et al.*, 2018). Furthermore, male *T. granifera* were found to be absent from the samples during the present study, thus the snail populations rely on parthenogenic reproduction (clones of their female parents) that results in low genetic diversity. Although Chaniotis *et al.* (1980b) reported male *T. granifera* from its invasive ranges in Puerto Rico, Veeravechskij *et al.* (2018b)

did not find any evidence of males within the populations in its native ranges in Thailand. In addition, all other studies in South Africa have also demonstrated that no males were present in any of the studied populations (Appleton & Nadasan, 2002; Appleton *et al.*, 2009; Miranda *et al.*, 2011a, 2011b). Thus, new-born *T. granifera* are genetically identical (clones) to their female parent (Appleton *et al.*, 2009). Parthenogenic reproduction may have some advantages for *T. granifera*, as only a single individual is able to start a new population (Smith, 1971; Jokela *et al.*, 1997; Gerritsen, 1980; Ben-Ami & Heller, 2007; Miranda *et al.*, 2011b), driving its success as an invader (Appleton *et al.*, 2009). Furthermore, asexual populations have been found to have a reduced risk of parasitic infection and fewer predators that feed on them (Lively, 2001; Ben-Ami & Heller, 2005; Miranda *et al.*, 2011b). According to the “Genetic Paradox of Invasions” hypothesis, asexual reproduction and high reproductive output of invasive species enable them to avoid the risk of reducing genetic drift and inbreeding (Schrieber & Lachmuth, 2017). Clonal reproduction also gives *T. granifera* another advantage that contributes to the successful invasion and expansion of the snail. Since all sexually mature individuals can contribute to population growth, *T. granifera* is able to rapidly colonise new environments and can quickly reach high densities (Baker, 1955; Maynard-Smith, 1978; Veeravechsukij *et al.*, 2018b).

Although Appleton *et al.* (2009) reported two distinct morphological variants of *T. granifera* in KwaZulu-Natal, it is possible that they may be genetically identical. However, this will need to be confirmed with further analyses, as the present study did not assess different morphological variants. Aquatic snails have been found to have morphological variability due to different environmental conditions across different habitats that can produce considerable shell modifications (Dillon, 2000; Glaubrecht, 2004, 2009, 2011; Veeravechsukij *et al.*, 2018b). Variations in features such as shell colour, size and shape do not always allow for precise identifications and do not always indicate different species and/or morphs (Davis, 1994; Michel, 1994; Von Gersdorff Sørensen *et al.*, 2005; Veeravechsukij *et al.*, 2018b). A study by Veeravechsukij *et al.* (2018b) found that morphometric data of *T. granifera* from Thailand were insufficient in distinguishing between different morphs of *T. granifera*. In addition, the aforementioned study also found that two distinct morphs, based on molecular analysis, did not indicate any observable differences in shell morphology. This highlights the importance of using molecular techniques rather than only morphological techniques to identify genetic differences between individuals in order to distinguish genotypical diversity (Von Gersdorff Sørensen *et al.*, 2005; Veeravechsukij *et al.*, 2018b).

Results indicated that the *T. granifera* sequences from the present study were found to be identical to sequences of *T. granifera* from China and Thailand. This suggests that *T. granifera* was likely introduced to South Africa from China or Thailand, which further confirms as well as

narrows down previous reports that it was introduced from Southeast Asia (Appleton *et al.*, 2009; Appleton & Miranda, 2015). The results from the present study are in support of the statements of Appleton and Nadasan (2002), that *T. granifera* were likely introduced to South Africa via the aquarium trade from Hong Kong. The chances of aquatic invasive species introductions are increased through the aquarium trade (Walker, 1978; Madsen & Frandsen, 1989; Cowie, 1998; Pointier, 1999; Letelier *et al.*, 2007; Strayer, 2010; Preston *et al.*, 2022). Especially since the South African Revenue Service data indicated that imports from Asia have doubled during the period 1998 to 2011 (Appleton & Miranda, 2015), with particularly strong links between the aquarium trade and Hong Kong as a source of supply (Appleton & Nadasan, 2002). After the first collection of *T. granifera* in 1999 from South Africa, Appleton and Miranda (2015) reported two additional Asian freshwater snail species introduced into South Africa, *Radix rubiginosa* was collected in 2004 and *Gyraulus chinensis* in 2006.

Interestingly, the phylogenetic analysis during the present study indicated that *cox1* sequences of *T. granifera* from China and Thailand were identical. This may be as a result of introductions of invasive morphs between the two countries, however, this requires further investigation. A better understanding of the genetic diversity and phylogeny of aquatic snails will be useful in future studies to consider host and parasite relationships as well as their invasion dynamics (Ebbs *et al.*, 2018; Veeravechskij *et al.*, 2018b). For example, in Lake Malawi, Genner *et al.* (2008) found that a highly invasive morph of *M. tuberculata* introduced from Southeast Asia was free from trematode infections and that this invader was resistant to native trematodes that were found to infect the native morphs. In addition, the invasion of Asian morphs of *M. tuberculata* in Lake Malawi have been found to threaten the native thiarid species diversity in the region (Genner *et al.*, 2004; Genner *et al.*, 2007; Von Gersdorff Sørensen *et al.*, 2005). In another example, the invasion of *T. granifera* in the Caribbean islands have led to the extinction of some thiarid species while others have become scarce (Pointier *et al.*, 2010). These findings call attention to the importance of not only understanding the interactions between invasive and native snail species but also understanding the genetic diversity of these species which drives their ecological interactions and their relationships with parasites. These interactions can have severe effects on ecosystem function and community structures of native communities (Johnson *et al.*, 2009), especially since trematodes from snail hosts pose a threat to human and animal health (Sodeman, 1991; McKoy *et al.*, 2011; Veeravechskij *et al.*, 2018a, 2018b; Yin *et al.*, 2022).

6.5 Conclusion

This chapter of the study aimed to investigate the role of the invasive thiarid (*T. granifera*) versus the native thiarid (*M. tuberculata*) as hosts for digenean trematodes within the Limpopo River system and Phongolo River, as well as to investigate the genetic diversity of *T. granifera*. The results from this study indicated that the invasive, *T. granifera*, did not act as a host for trematode parasites within the study area, while the native, *M. tuberculata*, was found to be infected with two species of trematodes known to reduce snail population growth. This release from parasites that influence snail population growth enables *T. granifera* to reach higher densities and spread rapidly, possibly contributing to their advantage over native snails within the invaded ecosystems of South Africa. All *T. granifera* samples from the selected sites were found to be genetically identical females, thus supporting previous hypotheses that all *T. granifera* in South Africa come from few or even a single invasion event or a small population of introduced snails. Furthermore, this study indicated that *T. granifera* were likely introduced from China or Thailand which narrows down previous reports that it was introduced from Southeast Asia. These findings support that *T. granifera* were likely introduced from Hong Kong via the aquarium trade. Due to the fact that *T. granifera* serves as a host for diverse species of trematodes within and outside its native ranges, it would be wise to further investigate its role as a host for trematodes within all invaded habitats across southern Africa (including Mozambique, Zimbabwe, Botswana and Eswatini).

Chapter 7: Conclusions and recommendations

7.1 Conclusions

Aquatic invasive species pose a threat to native freshwater biodiversity on a global scale. Knowledge on the impacts of such species on native biota as well as their distribution within invaded ecosystems should be monitored as this may aid in the development and implementation of management and control plans that may prevent the spread and destruction of invaders on our native biota and their ecosystems. Several studies have suggested that the invasive snail, *Tarebia granifera*, have a high probability of causing environmental harm due to their ability to reach high densities and as they are often the dominant invertebrate species within invaded aquatic ecosystems. Furthermore, *T. granifera* are known to be the first intermediate host for prevalent and diverse species of disease causing trematodes within and outside of their native ranges. Consequently, there is a need to investigate the current distribution and densities of these invaders, their possible environmental impacts, and their potential as hosts for parasites in South Africa, especially in the conservation areas of the Mpumalanga and Limpopo provinces. This chapter will provide concluding remarks on the main research findings in relation to the study aims and hypotheses. It will also highlight the study limitations and provide recommendations for future research.

7.1.1 Current distribution, densities, and population structures of *T. granifera* in the Limpopo River system and Phongolo River of South Africa (**Chapter 3**)

The aim for this chapter was to investigate the current distribution, densities, and population size structures of *T. granifera* at selected sites on the Limpopo River system and Phongolo River of southern Africa. Research questions included whether there is a relationship between *T. granifera* density and selected water quality parameters, and if native snail diversity and distribution might be influenced by high *T. granifera* density.

Results found that the invasive *T. granifera* were the dominant species and had much higher densities than all other molluscs sampled, comprising 89% of all collected samples. Furthermore, the invasive snail was the only species sampled from all sites found to have molluscs, and the densities reached by *T. granifera* exceeded the densities reported from its native range. Furthermore, the present study found that the *T. granifera* had an average birth rate of 1.89 individuals per day, corresponding with previous reports on the high birth rate of this invader. In terms of *T. granifera* population structures, results indicated that higher densities and higher new-born recruitment occurred during spring. While fewer new-born, and more adult snails were sampled during autumn. However, four of the sites sampled in autumn also had high contributions

of new-born snails compared to other sites sampled during autumn. Adult *T. granifera* are known to have a higher tolerance for environmental factors such as dry periods, colder temperatures, limited food availability and increased salinity, causing lower reproduction in adults during unfavourable conditions. These findings supports previous theories from the literature that *T. granifera* likely responds to environmental conditions rather than season, by increasing reproductivity and consequently speeding up population recovery when conditions become favourable, which contributes to their high invasion success.

Following these findings, the first hypothesis stating that *T. granifera* will occur at densities greater than within their native range and population structures will reflect an established population with an even spread of individuals throughout the size classes is partly supported. *Tarebia granifera* did have densities greater than reported from their native range thus this part was supported. However, although *T. granifera* population structures reflected an established population, they did not have an even spread of individuals throughout the size classes as a result of population fluctuations during different seasons of sampling due to different environmental conditions. Therefore, the second part of the hypothesis was not supported.

Furthermore, the present study did not find any significant correlations between *T. granifera* density and any of the water quality variables, contradicting suggestions from previous reports that differences in *T. granifera* density is influences by differences in water pH, conductivity, and TDS (total dissolved solids). The present study suggests that increased conductivity and TDS may rather be a result of the high densities and movement behaviour of benthic snails such as *T. granifera* that cause bioturbation, resulting in the resuspension of salts and nutrients from the sediments. As such, the second hypothesis: *T. granifera* density are related to water quality parameters such as pH, conductivity and temperature was not supported.

Due to sampling limitations such as seasonality, location (study area) and a lack of regular sampling events over a longer period, as well as different environmental factors responsible for the changes in *T. granifera* population dynamics, it is difficult to reach concrete conclusions regarding the environmental drivers behind the population dynamics of *T. granifera*. Thus, future studies should investigate such environmental drivers from different aquatic ecosystems/habitats during different seasons of the year over longer periods of time in order to gain more information on how *T. granifera* populations react to environmental changes and determine the environmental factors that influence their densities as well as their population size structures.

Results from the present study further found evidence of three native aquatic snail species (*Bulinus tropicus*, *Bulinus forskalii* and a Planorbidae sp.) that seem to have disappeared from sampled ecosystems with high densities of *T. granifera*. Furthermore, historical records of snail

species from the sites sampled during the present study revealed that numerous aquatic snail species (including *Melanoides tuberculata* as well as several *Biomphalaria* sp. and *Bulinus* sp.) seem to have disappeared from the sites that were dominated by the invasive *T. granifera*. Therefore, high densities of *T. granifera* seem to have a negative effect on native snail density and diversity, especially since native snail diversity from the sampled systems was found to be much lower than previously reported in the literature. As a result, the third hypothesis of the study: high densities of invasive *T. granifera* is expected to correlate with low native mollusc densities and diversity, suggesting that the invader can displace native mollusc communities was supported. As the reason for these extinctions and losses in native mollusc biodiversity is not yet understood, further research is needed to understand the interactions and drivers between *T. granifera* and native mollusc species.

7.1.2 The effects of *T. granifera* on benthic diatom community structures (Chapter 4 & 5)

These chapters of the study aimed to determine the impacts of *T. granifera* on the aquatic community structures of benthic diatoms, firstly with an *in situ* study at selected sites on the Limpopo River system and Phongolo River, and secondly to corroborate the findings of the *in situ* study with an experimental microcosm study.

Due to limitations such as differences in environmental variables e.g., temperature, nutrient availability, pollutants, water flow, water quality as well as complex trophic interactions within *in situ* river ecosystems, it can be difficult to draw concrete conclusions in terms of the effects *T. granifera* has on benthic diatom community structures. However, results from the *in situ* study indicated that the high densities reached by *T. granifera* within the invaded ecosystems resulted in disrupted benthic diatom community structures with lower diversity. At high densities, *T. granifera* grazing and biology alter benthic diatom community structures, changing diatom species abundance as well as the dominance patterns of diatom species and functional groups. These changes in diatom community structures were likely a result of the high grazing pressure and bioturbation (movement through sediments) caused by the high densities of the invasive snail at these sites. At high densities, snail excretions and bioturbation are known to change the nutrient cycles within invaded aquatic ecosystems that in return have a strong impact on diatom biomass and their community structures. These changes in the availability of nutrients affect the food web dynamics and ecosystem functions within invaded ecosystems, leading to the dominance of nutrient tolerant diatom species such as *Gomphonema* and *Fragilaria* species.

In contrast, sites sampled with lower densities of *T. granifera* had higher benthic diatom diversity, more taxa, higher species richness and contributed to different diatom functional groups. The single site dominated by the native *M. tuberculata* also had a higher number of diatom taxa,

species diversity and richness compared to the sites that were dominated by the invasive *T. granifera*. These findings are in support of previous reports that *T. granifera* have a higher carrying capacity than *M. tuberculata* and much higher ingestion rates than other native snails, which have strong impacts on benthic biomass within invaded aquatic ecosystems. Furthermore, results indicated that at high densities, *T. granifera* grazing may also cause shifts in benthic diatom dominance patterns. Sites with higher densities of the invasive snail were dominated by diatom species (such as *Cocconeis*, *Gomphonema* and *Fragilaria*) with characteristics that enable them to resist *T. granifera* grazing pressure. In addition, results also indicated that *T. granifera* size may play a role in their diet preference. Sites sampled with high densities of smaller snails (juveniles), were found to have higher abundances of large diatom species in comparison with sites that had high densities of larger (adults) *T. granifera*. This is especially of concern as previous studies have reported that juvenile snails can consume more food by mass than adult snails, thus juvenile snails can have larger impacts on food resources than larger adult snails.

Similar to the findings of the *in situ* river study, the microcosm study indicated that *T. granifera* is likely to alter the structure and diversity of benthic diatom communities within invaded habitats. Microcosm treatments with high densities of *T. granifera* had lower diatom diversity as well as disrupted community structures and functionality. Whereas microcosm treatments without the invasive snail had diatom communities with higher species diversity, richness, and evenness. Even though a loss in replication within each of the treatment groups occurred, the results from this study suggest that *T. granifera* indeed had a negative impact on benthic diatom community structures. Such changes in diatom community structures and declines in diatom biodiversity will likely affect the food web dynamics within these invaded ecosystems, as changes in one trophic level could lead to disturbances and cascading effects on higher trophic level species, with unknown effects on the aquatic ecosystem function within these invaded ecosystems. As diatoms are known to be excellent bioindicators of water quality and pollution, these changes in diatom community structures as a result of high *T. granifera* density may lead to erroneous results when diatoms are used as bioindicators. This study provides further evidence that there is a need to control this invader to avoid further degradation of our native aquatic biodiversity and other freshwater ecosystems.

Following the abovementioned findings, the hypothesis for this study that high densities of *T. granifera* in an aquatic community will result in a decrease of benthic diatom diversity, disrupting benthic diatom community structures and functionality, was supported. Management and remediation of biological invasions in South Africa need a more complete understanding of these invaders' impact and the mechanisms through which they occur. Further studies are needed to gain more information on *T. granifera* and the biological interactions it has on native biota in South

Africa. This may aid in the development and implementation of action plans that may prevent the further spread and destruction of this invader on natural ecosystems and native biota.

7.1.3 *Tarebia granifera* as a host for digenean trematodes and its genetic diversity in the Limpopo and Phongolo River systems (**Chapter 6**)

This chapter of this study aimed to investigate the role of the invasive thiarid (*T. granifera*) versus the native thiarid (*M. tuberculata*) as hosts for digenean trematodes within the Limpopo and Phongolo River systems, and to determine the genetic diversity of *T. granifera* within the selected study area.

The present study found that the invasive thiarid (*T. granifera*) were free from trematode infection within the study area. This could be as a result of evolutionary adaptations such as immunity against native trematodes, or the snails may have been introduced without parasites, or invasive parasites might not have been able to survive in the newly introduced environment due a lack of suitable intermediate or definitive hosts. This study further provide the first report of *M. tuberculata* as an intermediate host for *Haplorchis* sp. and *Centrocestus* sp. in South Africa. These species of trematodes are known to be zoonotic, and have been reported to infect humans in China, Thailand and Korea. From the results of the present study, it is clear that the biodiversity of parasitic trematodes is understudied in their snail hosts from South Africa and that these thiarid snails act as hosts for zoonotic trematodes that pose a risk to public and veterinary health.

Furthermore, *Haplorchis* and *Centrocestus* trematodes are known to play a negative role in snail population growth as they can cause reduced reproductive output. Thus, as *T. granifera* is released from trematodes that influence population growth in South Africa, this may give these invaders an advantage over their native counterparts in terms of reproduction, especially since *M. tuberculata* are infected with such trematodes. This can have considerable effects on the population dynamics and competitive interactions between these species, as it will likely contribute to the spread of *T. granifera* and could enable them to quickly become the dominant species and further displace *M. tuberculata* from invaded ecosystems. The release from parasites as well as the several characteristics of *T. granifera* such as having high fecundity due to parthenogenic reproduction that enables them to reach high densities and spread rapidly, a lack of natural predators as well as the ability to release chemical cues that drive away other native snails contributes to their high invasion success within the aquatic ecosystems of South Africa.

The hypothesis from the present study that stated that *T. granifera* acts as a first intermediate host for medically and ecologically important trematodes within the invaded ecosystems sampled, which may spillover to native species was not supported. However, considering the parasite

“Spillback” hypothesis, the presence of disease-causing trematodes in the native thiarid species may pose severe risks for wildlife and human health if these trematodes spillback into *T. granifera* populations in the future.

Furthermore, considering that *T. granifera* are well known to be hosts for several disease-causing trematodes within and outside of their native ranges and since several studies have indicated that *T. granifera* replace our native snail species. It will be important that future studies continue to investigate its role as a host for trematodes within invaded habitats across South Africa, since *T. granifera* have a much wider range of distribution in the country than covered during the sampling events of this study (Phongolo and Limpopo River systems). Especially since such problems may become worse in the future due to their rapid spread and their ability to reach extremely high densities within invaded ecosystems. Such investigations will need to be conducted on a regular basis during different seasons as parasite infections may differ between season. This study is also in accordance with Malatji *et al.* (2021) that future experimental studies may further our understanding on the suitability of *T. granifera* and *M. tuberculata* as hosts for native and/or invasive trematode species in South Africa. Especially since these snails are known to be hosts for trematodes that pose a threat to human and animal health worldwide. Information on the distribution of medically and veterinary important parasites are essential in order to control and prevent the spread of disease. Consequently, there is a need for parasitology and malacology studies in order to gain a better understanding of the biodiversity, biology and evolutionary relationships of thiarid snail hosts and their trematodes.

Despite previous reports that two distinct morphological variants of *T. granifera* exist in South Africa based on variation in shell morphology, the present study found that all *T. granifera* samples from the selected sites on the Limpopo River system in the Limpopo province and Phongolo River in KwaZulu-Natal were genetically identical based on mitochondrial DNA (*cox1* gene). Similar to previous reports from South Africa, this study also found all dissected *T. granifera* to be females with embryos in their brood pouches, thus all *T. granifera* are clones of their female parent. The findings of the present study suggest that it is likely that all *T. granifera* in South Africa come from few or even a single invasion event. Furthermore, the phylogenetic analysis from this study indicated that *T. granifera* was likely introduced into South Africa from China or Thailand, as the sequences obtained from *T. granifera* during the present study were genetically identical to sequences from China and Thailand, this narrows down previous reports that it was introduced from Southeast Asia.

Consequently, the last hypothesis of the study: due to the absence of males and the parthenogenic reproduction of *T. granifera*, the snails will have a low genetic diversity in South Africa was supported. The present study contributed to a better understanding of the genetic

diversity and phylogeny of *T. granifera* in South Africa, such information could be useful in future studies to better understand the possible parasite-host relationships of *T. granifera*. Such studies may also contribute to the control and prevention of diseases causing trematodes that use thiarid snails as hosts.

7.2 Recommendations

This study propose the following recommendations and potential future research considerations:

- Conduct more regular sampling events, during different seasons, over a longer time period and during different environmental factors in order to gain insight on the ecological drivers that influence the densities and population size structures of *T. granifera* within invaded ecosystems. A better understanding on how their populations will react towards environmental changes can lead to the development, implementation and management of control strategies.
- Further investigation is needed to understand how *T. granifera* interacts with native snails that cause local extinctions and declines in native snail species abundances from invaded ecosystems.
- Further field and experimental studies are needed to understand the effects of *T. granifera* on not only diatom community structures but also macroinvertebrates and other aquatic biota. Experimental studies such as microcosms might provide better insight as the effects of environmental variables can be controlled in these systems. Such studies should include more replicates (at least 5) and investigate the effects of *T. granifera* at different densities and/or size classes.
- Haplotype network analysis on *T. granifera* may help to better understand the relationships among their DNA sequences from across the world.
- Due to the fact that *T. granifera* and *M. tuberculata* both serve as a host for diverse and prevalent species of trematodes within and outside its native ranges, further investigations on their role as hosts for trematodes across South Africa is needed. Such investigations will need to be conducted on a regular basis over different seasons, as some parasite infections may be season dependent.
- There is a need to describe the species of trematodes from *M. tuberculata* with further molecular and phylogenetic analyses.
- Thiarid snails (such as *T. granifera* and *M. tuberculata*) will be good candidates for future experimental studies on the suitability of snail hosts for native and/or invasive trematode

species in South Africa. This will contribute to our understanding of the biodiversity, biology and evolutionary relationships of snail hosts and their parasitic trematodes, which will be of great significance.

- There is a need to investigate the genetic diversity of *M. tuberculata* in South Africa, as it is not yet determined if invasive morphs of the snail exists for sure and if they may harbour invasive trematodes that may pose a health risk to humans and/or our native biota.
- Fatty acid and stable isotope studies within ecosystems invaded by *T. granifera* will also be of great significance in order to gain a better understanding of the trophic dynamics within these invaded systems.

This study highlights the importance of further research on the impacts of *T. granifera* on aquatic biodiversity and ecosystem function. Management and remediation of biological invasions in southern Africa are in need of a more complete understanding of *T. granifera*'s impacts and the mechanisms through which they occur. If not managed, *T. granifera* will continue to spread and in the near future, climate change will likely further contribute to the spread at an unprecedented rate and range. This will likely have devastating implications on a wide range of native and endemic aquatic biota. It is therefore essential to implement proper management strategies in order to prevent further degradation of invaded aquatic communities and ecosystem health.

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*** Indicates references that were not used in the main text but are found in the Appendix.**

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Appendices

Appendix A

Appendix A1: *In situ* water quality and water nutrient variables, Upstream and Downstream sites measured at the Department of Water and Sanitation gauging stations on the Phongolo River from 1972 to 2017. Upstream – Gauging station located above the Pongolapoort Dam, Downstream – Gauging station located below the dam wall. PHON-3 and PHON-4 sites' water quality data were obtained from De Necker (2019). Mean±SD, range in parentheses, n = number of samples.

Chemical water variables	PHON-1 (n=44)	PHON-2 (n=28)	PHON-3 (n=2)	PHON-4 (n=4)
Temperature (°C)	-	-	26.00±5.52 (22.1 – 29.9)	25.08±7.42 (18.7 – 35.8)
Dissolved Oxygen (mg/L)	-	-	6.51±2.53 (4.72 – 8.3)	6.71±2.53 (3.91 – 9.37)
Oxygen concentration (%)	-	-	9.35±22.13 (63.7 – 95)	77.78±28.45 (45.3 – 104.4)
EC (µS/cm)	555.1±176 (317.7 – 999.4)	302.8±39.44 (227 – 401.3)	618±91.92 (553 – 683)	535±147.8 (403 – 735)
pH	8.21±0.33 (7.44 – 8.69)	8.08±0.3 (7.17 – 8.45)	8.56±0.06 (8.52 – 8.6)	8.32±0.4 (7.79 – 8.65)
Cl ⁻ (mg/L)	46.63±18.13 (20.76 – 99.4)	22.68±4.93 (13.17 – 37.1)	52.5±13.44 (43 – 62)	58±12.88 (41 – 71)
SO ₄ ²⁻ (mg/L)	19.26±6.72 (7.43 – 35.25)	14.36±8.58 (6.45 – 56.2)	20.5±10.61 (13 – 28)	63±69.31 (21 – 143)
NO ₃ ⁻ (mg/L)	0.56±0.25 (0.13 – 1.09)	0.24±0.16 (0.04 – 0.71)	0.43±0.53 (0.05 – 0.8)	0.73±0.22 (0.5 – 1)
NH ₄ ⁺ (mg/L)	0.05±0.03 (0.02 – 0.14)	0.05±0.05 (0.02 – 0.27)	0.15±0.04 (0.12 – 0.18)	0.07±0.01 (0.06 – 0.09)
PO ₄ ³⁻ (mg/L)	0.03±0.02 (0.002 – 0.08)	0.02±0.01 (0.02 – 0.04)	0.77±1.04 (0.03 – 1.5)	0.64±1.1 (0.03 – 2.28)

Appendix A2: *In situ* water quality and water nutrient variables measured from sites sampled on the Olifants River in the Kruger National Park from September to October 2020 and April to May 2021. Mean±SD, range in parentheses, n= number of samples. * Indicates sites sampled in 2021.

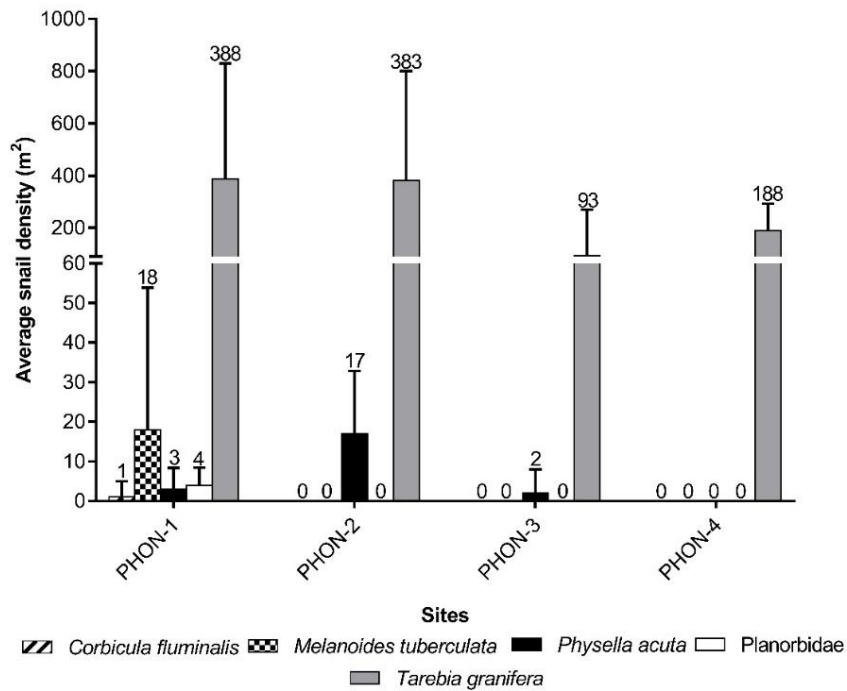
Chemical water variables	OLIF-1 (n=3)	OLIF-2 (n=3)	OLIF-3 (n=3)	OLIF-1* (n=3)	OLIF-2* (n=3)
Temperature (°C)	20.8	26.1	19	22.8	19.8
Dissolved Oxygen (mg/L)	9.30	7.79	8.14	11.42	10.10
Oxygen concentration (%)	107.36	98.48	89.11	127.9	106.1
TDS (mg/L)	520	994	640	907	387
EC (µS/cm)	742	860	580	1278	552
pH	8.44	8.25	7.95	8.73	8.60
Turbidity (FAU)	5.00±0.00 (5)	22.67±4.16 (18 – 26)	45.00±7.00 (40 – 53)	13±3 (10 – 16)	12±1.73 (10 -13)
COD (mg/L)	5.43±0.97 (4.6 – 6.5)	7.00±1.39 (6.2 – 8.6)	6.33±0.21 (6.1 – 6.5)	5.6±3.329 (3.2 – 9.4)	4.27±0.681 (3.5 – 4.8)
Cl ⁻ (mg/L)	37.00±2.00 (35 – 39)	36.00±0.00 (36)	20.27±1.15 (19.6 – 21.6)	26±2 (24 – 28)	32.67±0.58 (32 – 33)
SO ₄ ²⁻ (mg/L)	91.67±7.02 (85 – 99)	97.33±3.06 (94 – 100)	59.33±4.16 (56 – 64)	69.67±0.577 (69 – 70)	62.67±0.577 (62 – 63)
NO ₃ ⁻ (mg/L)	1.23±0.06 (1.2 – 1.3)	1.577±0.47 (1.2 – 2.1)	1.60±0.10 (1.5 – 1.7)	0.997±1.475 (0.14 – 2.7)	1.97±1.21 (0.6 – 2.9)
NO ₂ ⁻ (mg/L)	0.003±0.001 (0.002 – 0.003)	0.004±0.002 (0.003 – 0.006)	0.03±0.01 (0.03 – 0.04)	0.009±0.001 (0.008 – 0.01)	0.005±0.001 (0.004 – 0.006)
NH ₄ ⁺ (mg/L)	0.09±0.03 (0.06 – 0.12)	0.10±0.01 (0.09 – 0.11)	0.20±0.04 (0.17 – 0.24)	0.011±0.001 (0.011 – 0.012)	0.118±0.003 (0.115 – 0.118)
PO ₄ ³⁻ (mg/L)	0.23±0.06 (0.16 – 0.28)	0.26±0.14 (0.17 – 0.42)	0.64±0.11 (0.7 – 0.51)	0.008±0.005 (0.002 – 0.012)	0.107±0.081 (0.02 – 0.18)

Appendix A3: *In situ* water quality and water nutrient variables measured from sites sampled on the Limpopo River system between April and May 2021. Mean±SD, range in parentheses, n= number of samples.

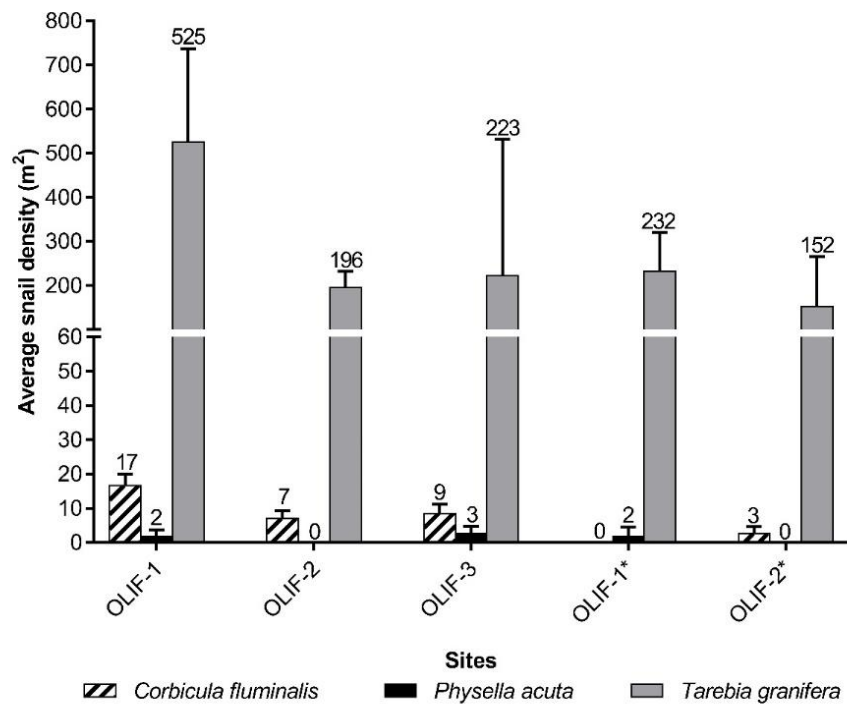
Chemical water variables	CROC (n=3)	LIMP-1 (n=3)	MATL (n=3)	LEPH (n=3)	LIMP-2 (n=3)	MOGA (n=3)	LIMP-3 (n=3)	SAND (n=3)	LUVU (n=3)	G-LETA (n=3)	LETA (n=3)	SHIN (n=3)
Temperature (°C)	21.6	21.3	22.3	21.7	22.2	22.4	25.4	26.4	24.4	22.4	21.2	22
Dissolved Oxygen (mg/L)	12.52	10.73	9.28	9.72	10.32	8.14	10.84	15.78	10.22	10.73	9.3	9.68
Oxygen concentration (%)	138.3	122.2	107.5	108.8	116	94.3	129.6	189.7	122.6	121.8	103.6	106.7
TDS (mg/L)	552	536	539	590	763	1500	287	1750	262	679	259	648
EC (µS/cm)	792	779	7380	855	1095	2160	421	2580	381	1001	378	931
pH	9.19	8.74	7.14	7.68	8.60	7.80	8.73	8.58	8.30	8.17	7.98	8.12
Turbidity (FAU)	35±3.61 (31 – 38)	20±1 (19 – 21)	4.67±2.31 (2 – 6)	7.3±0.58 (7 – 8)	9.67±7.09 (2 -16)	8±3 (5 – 11)	15.33±4.16 (12 – 20)	13.67±2.89 (12 – 17)	8±1.73 (7 – 10)	11.67±1.53 (10 – 13)	12.67±2.89 (11 – 16)	17.67±2.08 (16 – 20)
COD (mg/L)	12.97±3.56 (9.1 – 16.1)	9.9±0.7 (9.4 – 10.7)	8.67±2.538 (6.6 – 11.5)	5.2±2.5 (2.7 – 7.7)	11.6±0.781 (11.1 – 12.5)	14.77±3.102 (11.6 – 17.8)	17.27±20.56 (5.1 – 41)	18.6±1.51 (17 – 20)	5.33±3.436 (3.3 – 9.3)	5.97±3.581 (3.8 – 10.1)	7.03±2.732 (4 – 9.3)	9.87±1.629 (8 – 11)
Cl ⁻ (mg/L)	92.67±2.08 (91 – 95)	81.3±9.07 (71 – 88)	10.4±1.14 (9.6 – 11.7)	10.67±3.79 (8 – 15)	53±8.72 (47 – 63)	603±90.15 (516 – 696)	48±2 (46 – 50)	444±49.57 (387 – 477)	13.37±0.31 (13.1 – 13.7)	19.33±2.52 (17 – 22)	28.33±8.39 (23 – 38)	87.33±0.58 (87 – 88)
SO ₄ ²⁻ (mg/L)	61±1 (60 -62)	58±0.0 (58)	8.67±0.577 (1 – 2)	4.67±1.15 (4 – 6)	14±1.73 (12 – 15)	216.67±65.26 (155 – 285)	33.33±2.08 (31 – 35)	133±3.46 (129 – 135)	4±1 (3 – 5)	14.67±3.79 (12- 19)	13±1 (12 – 14)	12±1 (11 -13)

Appendix A3 continued.

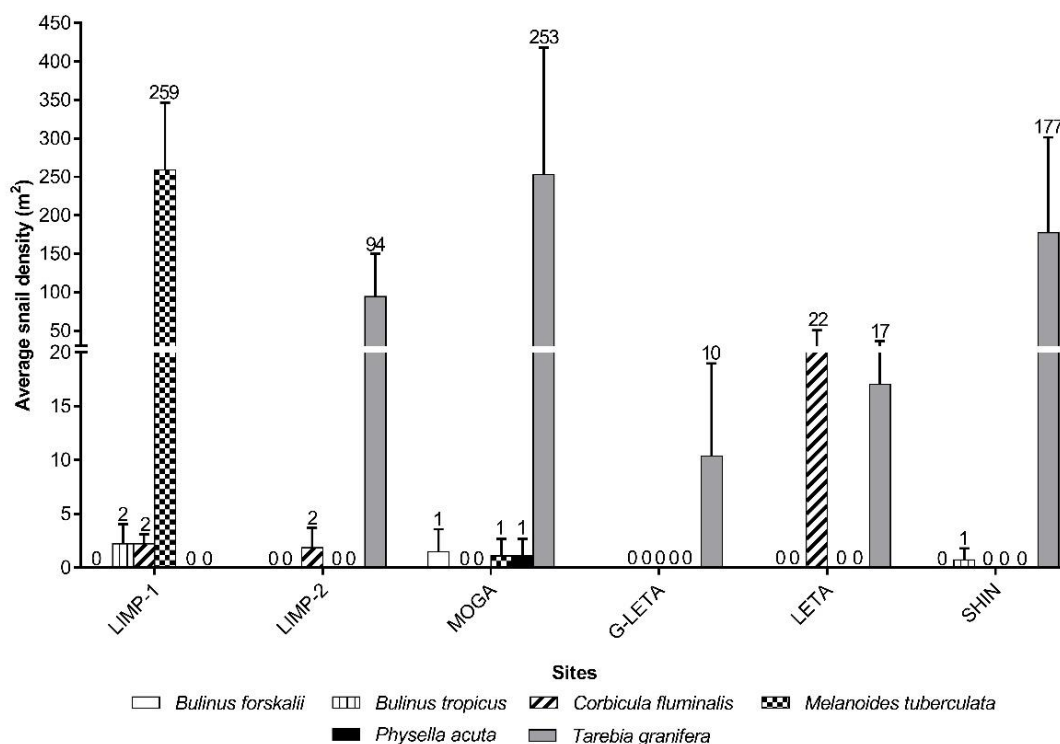
Chemical water variables	CROC (n=3)	LIMP-1 (n=3)	MATL (n=3)	LEPH (n=3)	LIMP-2 (n=3)	MOGA (n=3)	LIMP-3 (n=3)	SAND (n=3)	LUVU (n=3)	G-LETA (n=3)	LETA (n=3)	SHIN (n=3)
NO₃⁻ (mg/L)	1.3±0.781 (0.8 – 2.2)	1.33±0.67 (0.9 – 2.1)	2.4±0.246 (2.2 – 2.8)	0.9±0.346 (0.7 – 1.3)	1.767±0.404 (1.4 – 2.2)	0.93±0.115 (0.8 – 1)	0.83±0.404 (0.6 – 1.3)	1.63±0.252 (1.4 – 1.9)	0.407±0.101 (0.3 – 0.5)	0.203±0.152 (0.028 – 2.4)	0.27±0.289 (0.06 – 0.6)	0.87±0.231 (0.6 – 1)
NO₂⁻ (mg/L)	0.015±0.002 (0.013 – 0.016)	0.016±0.001 (0.015 – 0.016)	0.013±0.007 (0.005 – 0.018)	0.005±0.002 (0.003 – 0.006)	0.081±0.103 (0.02 – 0.2)	0.005±0.004 (0.002 – 0.009)	0.008±0.001 (0.007 – 0.009)	0.023±0.01 (0.011- 0.027)	0.005±0.003 (0.002 – 0.008)	0.008±0.001 (0.008 – 0.009)	0.009±0.002 (0.006 – 0.01)	0.005±0.002 (0.004 – 0.007)
NH₄⁺ (mg/L)	0.083±0.006 (0.08 – 0.09)	0.043±0.006 (0.04 – 0.05)	0.073±0.012 (0.06 – 0.08)	0.0147±0.002 (0.013 – 0.016)	0.06±0.01 (0.05 – 0.07)	0.137±0.127 (0.04 – 0.28)	0.177±0.006 (0.17 – 0.18)	0.127±0.064 (0.09 – 0.2)	0.096±0.002 (0.095 – 0.098)	0.052±0.022 (0.027 – 0.07)	0.0153±0.001 (0.014 – 0.016)	0.18±0.0 (0.18)
PO₄³⁻ (mg/L)	0.153±0.006 (0.15 – 0.16)	0.04±0.017 (0.02 – 0.05)	0.054±0.039 (0.012 – 0.09)	0.461±0.324 (0.185 – 0.818)	0.237±0.284 (0.03 – 0.56)	0.161±0.011 (0.15 – 0.171)	0.097±0.011 (0.085 – 0.107)	0.233±0.3 (0.06 – 0.579)	0.104±0.061 (0.033 – 0.14)	0.03±0.01 (0.02 – 0.04)	0.016±0.006 (0.011 – 0.022)	0.05±0.036 (0.02 – 0.09)



Appendix A4: Average dead snail (empty shells) population density ($\pm$ SD) sampled from the four sites on the Phongolo River between August and September 2017.



Appendix A5: Average dead snail (empty shells) population density ($\pm$ SD) sampled from the three sites on the Olifants River inside the Kruger National Park between September to October 2020 and April to May 2021.



Appendix A6: Average dead snail (empty shells) population density ($\pm$ SD) sampled from the seven sites on the Limpopo River system between April to May 2021.

Appendix A7: List of aquatic snail species historically recorded from the river systems sampled during the present study.

River	Aquatic snails	Date	Reference
Phongolo	<i>Anisus natalensis</i> (Krauss, 1848)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Aspatharia petersi</i> (von Martens, 1860).	1971	Pretorius <i>et al.</i> , 1975.
	<i>Aspatharia wahlbergi</i> (Krauss, 1848)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Bellamya capillata</i> (Frauenfeld, 1865)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Biomphalaria pfeifferi</i> (Krauss, 1848)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Bulinus aricanus</i> (Krauss, 1848)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Bulinus forskalii</i> (Ehrenberg, 1831)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Bulinus natalensis</i> (Küster, 1841)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Cleopatra ferruginea</i> (Lea & Lea, 1851)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Corbicula africana</i> (Krauss, 1848)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Eupera ferruginea</i> (Krauss 1848)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Ferrissia cawstonii</i> (Walker, 1903)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Gyraulus costulatus</i> (Krauss, 1848)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Lanistes ovum</i> (Peters, 1845)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Lymnaea columella</i> (Say, 1817)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Lymnaea natalensis</i> (Krauss, 1848)	1966	Oberholzer & Van Eeden, 1967.
	<i>Melanoides tuberculata</i> (Müller, 1774)	1966	Oberholzer & Van Eeden, 1967.
	<i>Pisidium pirothi</i> (Jickeli, 1881)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Segmentorbis kanisaensis</i> (Preston, 1914)	1971	Pretorius <i>et al.</i> , 1975.
	<i>Unio mossambicensis</i> (Martens, 1860)	1971	Pretorius <i>et al.</i> , 1975.

Appendix A7 continued.

River	Aquatic snails	Date	Reference
Crocodile	<i>Ancylidae</i> (Rafinesque, 1815)	2016	FBIS, 2020.
	<i>Anisus</i> (Studer, 1820)	1957	FBIS, 2020.
	<i>Bulinus</i> (Müller, 1781)	2016	FBIS, 2020.
	<i>Burnupia</i> (Walker, 1912)	1964	FBIS, 2020.
	<i>Cafferia caffra</i> (Krauss, 1848)	-	FBIS, 2020.
	<i>Corbicula fluminalis</i> (Müller, 1774)	2015	FBIS, 2020.
	<i>Ferrissia</i> (Walker, 1903)	1964	FBIS, 2020.
	<i>Gyraulus</i> (Charpentier, 1837)	1964	FBIS, 2020.
	<i>Lymnaea natalensis</i> (Krauss, 1848)	1957	FBIS, 2020.
	<i>Physella acuta</i> (Draparnaud, 1805)	2016	FBIS, 2020.
	<i>Planorbis</i> (Müller, 1773)	1965	FBIS, 2020.
	<i>Sphaeriidae</i> (Deshayes, 1855)	2016	FBIS, 2020.
	<i>Sphaerium capense</i> (Krauss, 1848)	2016	FBIS, 2020.
	<i>Tarebia granifera</i> (Lamarck, 1816)	2017	FBIS, 2020.
	<i>Unio caffer</i> (Krauss, 1848)	1966	Oberholzer & Van Eeden, 1967.
Limpopo	<i>Biomphalaria pfeifferi</i> (Krauss, 1848)	1962	FBIS, 2020.
	<i>Bulinus</i> (Müller, 1781)	1962	FBIS, 2020.
	<i>Corbicula</i> (Megerle von Mühlfeld, 1811)	1962	FBIS, 2020.
	<i>Ferrissia</i> (Walker, 1903)	1962	FBIS, 2020.
	<i>Gyraulus</i> (Charpentier, 1837)	1962	FBIS, 2020.
	<i>Lymnaea</i> (Lamarck, 1799)	1962	FBIS, 2020.
	<i>Melanoides tuberculata</i> (Müller, 1774)	1962	FBIS, 2020.
	<i>Pisidium</i> (Pfeiffer, 1821)	1962	FBIS, 2020.
	<i>Venus lamellaris</i> (Schumacher, 1817)	-	FBIS, 2020.
Matlabas			No data found.
Lephalala	<i>Biomphalaria</i> (Preston, 1910)	1960	FBIS, 2020.
Mogalakwena	<i>Biomphalaria pfeifferi</i> (Krauss, 1848)	1960	FBIS, 2020.
	<i>Bulinus</i> (Müller, 1781)	1960	FBIS, 2020.
Sand	<i>Lymnaea</i> (Lamarck, 1799)	2015	FBIS, 2020.
	<i>Sphaeriidae</i> (Deshayes, 1855)	2016	FBIS, 2020.
	<i>Bulinus</i> (Müller, 1781)	2016	FBIS, 2020.
	<i>Corbicula</i> (Megerle von Mühlfeld, 1811)	2016	FBIS, 2020.
	<i>Physella acuta</i> (Draparnaud, 1805)	2016	FBIS, 2020.
	<i>Tarebia granifera</i> (Lamarck, 1816)	2017	FBIS, 2020.
Luvuvhu	<i>Bulinus</i> (Müller, 1781)	2016	FBIS, 2020.
	<i>Burnupia</i> (Walker, 1912)	2017	FBIS, 2020.
	<i>Ceratophallus natalensis</i> (Krauss, 1848)	2015	FBIS, 2020.
	<i>Corbicula</i> (Megerle von Mühlfeld, 1811)	2017	FBIS, 2020.
	<i>Lymnaea</i> (Lamarck, 1799)	2016	FBIS, 2020.
	<i>Physella acuta</i> (Draparnaud, 1805)	2016	FBIS, 2020.
	<i>Planorbidae</i> (Rafinesque, 1815)	2016	FBIS, 2020.
	<i>Sphaeriidae</i> (Deshayes, 1855)	2017	FBIS, 2020.
	<i>Sphaerium capense</i> (Krauss, 1848)	2016	FBIS, 2020.
	<i>Tarebia granifera</i> (Lamarck, 1816)	2017	FBIS, 2020.
Olifants	<i>Biomphalaria</i> (Preston, 1910)	1965	FBIS, 2020.
	<i>Bulinus</i> (Müller, 1781)	2015	FBIS, 2020.
	<i>Burnupia</i> (Walker, 1912)	1965	FBIS, 2020.
	<i>Corbicula africana</i> (Krauss, 1848)	1959	FBIS, 2020.
	<i>Corbicula fluminalis</i> (Krauss, 1848)	2017	FBIS, 2020.
	<i>Eupera</i> (Bourguignat, 1854)	1986	FBIS, 2020.
	<i>Ferrissia</i> (Walker, 1903)	1965	FBIS, 2020.
	<i>Gyraulus costulatus</i> (Krauss, 1848)	1960	FBIS, 2020.
	<i>Lymnaea</i> (Lamarck, 1799)	1965	FBIS, 2020.
	<i>Melanoides tuberculata</i> (Müller, 1774)	2015	FBIS, 2020.
	<i>Physella acuta</i> (Draparnaud, 1805)	2015	FBIS, 2020.
	<i>Pisidium</i> (Pfeiffer, 1821)	2016	FBIS, 2020.
	<i>Planorbidae</i> (Rafinesque, 1815)	2017	FBIS, 2020.
	<i>Sphaeriidae</i> (Deshayes, 1855)	2016	FBIS, 2020.
	<i>Tarebia granifera</i> (Lamarck, 1816)	2017	FBIS, 2020.
	<i>Unio caffer</i> (Krauss, 1848)	1966	Oberholzer & Van Eeden, 1967.
Great Letaba	<i>Eupera ferruginea</i> (Krauss, 1848)	1966	Oberholzer & Van Eeden, 1967.

Appendix A7 continued.

River	Aquatic snails	Date	Reference
Letaba	<i>Aplexa marmorata</i> (Guilding, 1828)	2006	Wolmarans & de Kock, 2006
	Ampullariidae (Gray, 1824)	2017	FBIS, 2020.
	Ancylidae (Walker, 1923)	2016	FBIS, 2020.
	<i>Aspatharia wahlbergi</i> (Krauss, 1848)	1932	FBIS, 2020.
	<i>Biomphalaria pfeifferi</i> (Krauss, 1848)	2017	FBIS, 2020.
	<i>Bulinus</i> (Müller, 1781)	2016	FBIS, 2020.
	<i>Bulinus forskali</i> (Ehrenberg, 1831)	2001	Wolmarans & de Kock, 2006
	<i>Burnupia</i> (Walker, 1912)	2016	FBIS, 2020.
	<i>Corbicula fluminalis</i> (O.F. Müller, 1774)	2017	FBIS, 2020.
	<i>Corbicula natalensis</i> (Cessin, 1879)	1935	FBIS, 2020.
	Etheriidae (Deshayes, 1830)	2016	FBIS, 2020.
	<i>Lanistes ovum</i> (Peters, 1845)	1935	Wolmarans & de Kock, 2006
	<i>Lymnaea natalensis</i> (Krauss, 1848)	2015	FBIS, 2020.
	<i>Lymnaea columella</i> (Say, 1817)	2006	Wolmarans & de Kock, 2006
	<i>Melanoides tuberculata</i> (Müller, 1774)	2006	Wolmarans & de Kock, 2006
	<i>Melanoides victoriae</i> (Dohrn, 1865)	2006	Wolmarans & de Kock, 2006
	Neritidae (Rafinesque, 1815)	2016	FBIS, 2020.
	<i>Physella acuta</i> (Draparnaud, 1805)	2017	FBIS, 2020.
	<i>Pisidium</i> (Pfeiffer, 1821)	2016	FBIS, 2020.
	Planorbidae (Rafinesque, 1815)	2015	FBIS, 2020.
	Sphaeriidae (Deshayes, 1855)	2016	FBIS, 2020.
	<i>Tarebia granifera</i> (Lamarck, 1816)	2017	FBIS, 2020.
	<i>Unio caffer</i> (Krauss, 1848)	2016	FBIS, 2020.
Shingwedzi	<i>Lanistes ovum</i> (Peters, 1845)	1966	Oberholzer & Van Eeden, 1967.

Appendix B

Appendix B1: List of diatom species sampled from the selected sites on the Phongolo River between August and September 2017.

Species	Colonisation strategy	Group	Size class	Life-forms			Ecological guilds			CEMFG's	Sites							
				Mobile	Pioneer	Colonial	High profile	Low profile	Motile		PHON-1		PHON-2		PHON-3		PHON-4	
											µ-Count	%	µ-Count	%	µ-Count	%	µ-Count	%
<i>Achnanthidium</i> sp. (Kützing)	Pioneer	Monoraphideae	1	1	0	0	0	1	0	LS1	0	0	0	0	0	0	18	4.50
<i>Amphora coffeaeformis</i> (Agardh) Kützing	Intermediate	Biraphideae	5	1	0	0	0	1	0	LS5	21	5.25	0	0	0	0	0	0
<i>Aulacoseira granulata</i> (Ehr.) Simonsen	Pioneer	Centric	5	0	0	1	0	0	0	-	88	22.00	85	21.25	0	0	0	0
<i>Capartogramma crucicula</i> (Grunow ex Cleve) Ross	Pioneer	Biraphideae	4	1	0	0	0	0	1	MS4	7	1.75	0	0	0	0	0	0
<i>Cocconeis placentula</i> (Ehrenberg) Heinzerling	Intermediate	Monoraphideae	5	1	0	0	0	1	0	LS5	89	22.25	60	15.00	53	13.25	117	29.25
<i>Craticula halophila</i> (Grunow) DG Mann	Late	Biraphideae	3	1	0	0	0	0	1	MS3	0	0	0	0	19	4.75	0	0
<i>Cyclotella meneghiniana</i> Kützing	Pioneer	Centric	4	0	0	0	0	0	0	-	20	5.00	0	0	19	4.75	0	0
<i>Encyonema silesiacum</i> (Bleisch in Rabh.) D.G. Mann	Late	Biraphideae	4	1	0	1	1	0	0	HS4	0	0	15	3.75	0	0	0	0
<i>Eolimna subminuscula</i> (Manguin) Moser Lange-Bertalot & Metzeltin	Intermediate	Biraphideae	2	1	0	0	0	0	1	MS2	0	0	0	0	12	3.00	0	0
<i>Fragilaria tenera</i> (W. Smith) Lange-Bertalot	Pioneer	Araphideae	2	0	0	1	1	0	0	HS2	0	0	0	0	0	0	25	6.25
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing)	Pioneer	Araphideae	3	0	0	1	1	0	0	HS3	33	8.25	10	2.50	0	0	24	6.00
Σ- <i>Fragilaria</i> sp.												8.25		2.50		0		12.25
<i>Gomphonema affine</i> Kützing	Intermediate	Biraphideae	4	1	0	0	1	0	0	HS4	0	0	39	9.75	0	0	0	0
<i>Gomphonema minutum</i> (Agardh) Agardh	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	0	0	22	5.50	0	0	0	0
<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	12	3.00	0	0	0	0	0	0
<i>Gomphonema</i> sp. Ehrenberg	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	22	5.50	0	0	23	5.75	14	3.50
Σ- <i>Gomphonema</i> sp.												8.50		15.25		5.75		3.50
<i>Gyrosigma attenuatum</i> (Kützing) Rabenhorst	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	0	0	17	4.25	17	4.25	0	0
<i>Gyrosigma</i> sp. Hassall	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	12	3.00	0	0	0	0	0	0
Σ- <i>Gyrosigma</i> sp.												3		4.25		4.25		0
<i>Hippodonta</i> sp. (Lange-Bertalot) Metzeltin & Witkowski	Late	Biraphideae	3	1	0	0	0	0	1	MS3	0	0	0	0	15	3.75	0	0
<i>Mastogloia smithii</i> Thwaites	Pioneer	Biraphideae	4	1	0	0	0	0	1	MS4	0	0	8	2.00	0	0	0	0

<i>Navicula erifuga</i> Lange-Bertalot	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	0	0	59	14.75	19	4.75	27	6.75
<i>Navicula radiosa</i> Kützing	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	22	5.50	0	0	0	0	0	0
<i>Navicula rostellata</i> Kützing	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0	0	0	0	0	29	7.25
<i>Navicula</i> sp. Bory de St. Vincent	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	26	6.50	0	0	33	8.25	51	12.75
<i>Navicula veneta</i> Kützing	Intermediate	Biraphideae	2	1	0	0	0	0	1	MS2	0	0	15	3.75	17	4.25	0	0
Σ-Navicula sp.												12		18.5		17.25		26.75
<i>Nitzschia dissipata</i> (Kützing) Grunow	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0	9	2.25	26	6.50	0	0
<i>Nitzschia filiformis</i> (W.M. Smith) Van Heurck	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0	0	0	22	5.50	0	0
<i>Nitzschia palea</i> (Kützing) W. Smith	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	18	4.50	13	3.25	18	4.50	0	0
<i>Nitzschia recta</i> Hantzsch	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0	0	0	20	5.00	0	0
<i>Nitzschia sigma</i> (Kützing) W Smith	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	0	0	0	0	19	4.75	0	0
<i>Nitzschia</i> sp. Hassall	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	30	7.50	15	3.75	25	6.25	46	11.50
<i>Nitzschia siliqua</i> Archibald	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	0	0	0	0	0	0	28	7.00
Σ-Nitzschia sp.												12		7		32.50		18.50
<i>Rhopalodia gibberula</i> (Ehrenberg) O Müller	Pioneer	Biraphideae	4	1	0	0	0	0	1	MS4	0	0	20	5.00	0	0	0	0
<i>Seminavis strigosa</i> (Hustedt) Danieleadis & Economou-Amilli	Intermediate	Biraphideae	3	1	0	0	0	1	0	LS3	0	0	13	3.25	0	0	0	0
<i>Tryblionella apiculata</i> Gregory	Late	Biraphideae	4	1	0	0	0	0	1	MS4	0	0	0	0	19	4.75	21	5.25
<i>Tryblionella levidensis</i> W Smith	Late	Biraphideae	4	1	0	0	0	0	1	MS4	0	0	0	0	24	6.00	0	0
Σ-Tryblionella sp.												0		0		10.75		5.25
% Diatom Community Characterisation											PHON-1		PHON-2		PHON-3		PHON-4	
Colonisation strategy								Pioneer colonists			40		35		9		16.75	
								Intermediate colonists			60		61.25		71.75		78	
								Late colonists			0		3.75		19.25		5.25	
Group								Monoraphideae			22.25		15		13.25		33.75	
								Biraphideae			42.5		61.25		82		54	
								Centric			27		21.25		4.75		0	
								Araphideae			8.25		2.5		0		12.25	
Size class								Size 1			0		0		0		4.50	
								Size 2			0		3.75		7.25		6.25	
								Size 3			28.75		33		29.75		34.75	
								Size 4			13.25		22.75		40.75		25.25	
								Size 5			58		40.50		22.25		29.25	
Life-forms								Mobile			68.16		73.49		100		93.60	
								Pioneer			0		0		0		0	
								Colonial			31.84		26.51		0		6.40	
Ecological guilds								High profile			13.13		24.92		6.04		3.99	
								Low profile			42.47		23.93		13.91		38.46	
								Motile			44.40		51.15		80.05		57.55	
CEMFG's								HS1			0		0		0		0	
								HS2			0		0		0		0	

	HS3	13.13	7.21	6.04	3.99
	HS4	0	17.70	0	0
	HS5	0	0	0	0
	LS1	0	0	0	5.13
	LS2	0	0	0	0
	LS3	0	4.26	0	0
	LS4	0	0	0	0
	LS5	42.47	19.67	13.91	33.33
	MS1	0	0	0	0
	MS2	0	4.92	7.61	0
	MS3	18.53	28.52	25.20	28.77
	MS4	12.74	12.13	37.80	28.77
	MS5	13.13	5.57	9.45	0

Appendix B2: List of diatom species sampled from the selected sites on the Olifants River inside the Kruger National Park between September to October 2020 and April to May 2021. * Indicates sites sampled in 2021.

Species	Colonisation strategy	Group	Size class	Life-forms			Ecological guilds			CEMFG's	Sites									
				Mobile	Pioneer	Colonial	High profile	Low profile	Motile		OLIF-1		OLIF-2		OLIF-3		OLIF-1*		OLIF-2*	
											µ-Count	%	µ-Count	%	µ-Count	%	µ-Count	%	µ-Count	%
<i>Achnanthidium eutrophilum</i> (Lange-Bertalot) Lange-Bertalot	Pioneer	Monoraphideae	1	1	0	0	0	1	0	LS1	0	0.00	0	0.00	4	0.91	0	0.00	0	0.00
<i>Achnanthidium exiguum</i> (Grunow) Czamecki	Pioneer	Monoraphideae	1	1	0	0	0	1	0	LS1	7	1.85	2	0.49	0	0.00	2	0.57	0	0.00
Σ- <i>Achnanthidium</i> sp.												1.85		0.49		0.91		0.57		0
<i>Amphora coffeaeformis</i> (Agardh) Kützing	Intermediate	Biraphideae	5	1	0	0	0	1	0	LS5	32	8.80	13	3.27	4	1.07	7	1.80	7	1.77
<i>Amphora fontinalis</i> Hustedt	Intermediate	Biraphideae	5	1	0	0	0	1	0	LS5	8	2.22	0	0.00	0	0.00	0	0.00	0	0.00
<i>Amphora montana</i> Krasske	Intermediate	Biraphideae	5	1	0	0	0	1	0	LS5	5	1.48	0	0.00	0	0.00	0	0.00	0	0.00
<i>Amphora pediculus</i> (Kützing) Grunow	Intermediate	Biraphideae	5	1	0	0	0	1	0	LS5	4	1.11	0	0.00	4	1.07	0	0.00	0	0.00
<i>Amphora strigosa</i> Hustedt	Intermediate	Biraphideae	5	1	0	0	0	1	0	LS5	0	0.00	0	0.00	0	0.00	1	0.25	1	0.24
<i>Amphora veneta</i> Kützing	Intermediate	Biraphideae	5	1	0	0	0	1	0	LS5	6	1.76	0	0.00	2	0.50	1	0.33	0	0.00
Σ- <i>Amphora</i> sp.												15.37		3.27		2.64		2.38		2.01
<i>Caloneis hyalina</i> Hustedt	Late	Biraphideae	3	1	0	0	0	0	1	MS3	0	0.00	0	0.00	7	1.82	0	0.00	0	0.00
<i>Caloneis molaris</i> (Grunow) Krammer	Late	Biraphideae	3	1	0	0	0	0	1	MS3	0	0.00	0	0.00	0	0.00	7	1.72	0	0.00
<i>Caloneis silicula</i> (Ehrenberg) Cleve	Late	Biraphideae	3	1	0	0	0	0	1	MS3	1	0.37	0	0.00	0	0.00	0	0.00	0	0.00
Σ- <i>Caloneis</i> sp.												0.37		0		1.82		1.72		0
<i>Cocconeis pediculus</i> Ehrenberg	Intermediate	Monoraphideae	5	1	0	0	0	1	0	LS5	4	1.02	10	2.37	12	2.89	3	0.65	0	0.00
<i>Cocconeis placentula</i> Ehrenberg	Intermediate	Monoraphideae	5	1	0	0	0	1	0	LS5	73	20.37	85	20.95	135	33.47	20	4.98	24	5.78
Σ- <i>Cocconeis</i> sp.												21.39		23.32		36.36		5.63		5.78
<i>Craticula buderii</i> (Hustedt) Lange-Bertalot	Late	Biraphideae	2	1	0	0	0	0	1	MS2	18	5.09	17	4.09	10	2.40	32	7.92	11	2.57
<i>Craticula halophila</i> (Grunow ex Van Heurck) Mann	Late	Biraphideae	3	1	0	0	0	0	1	MS3	3	0.93	0	0.00	0	0.00	9	2.29	14	3.29
Σ- <i>Craticula</i> sp.												6.02		4.09		2.40		10.21		5.86

<i>Cymbella aspera</i> (Ehrenberg) H Peragallo	Intermediate	Biraphideae	5	1	0	0	1	0	0	HS5	0	0.00	3	0.65	0	0.00	15	3.68	10	2.33
<i>Cymbella kolbei</i> Hustedt	Intermediate	Biraphideae	5	1	0	0	1	0	0	HS5	0	0.00	0	0.00	0	0.00	37	9.15	0	0.00
<i>Cymbella neocistula</i> Krammer	Intermediate	Biraphideae	5	1	0	0	1	0	0	HS5	0	0.00	0	0.00	0	0.00	21	5.23	0	0.00
<i>Cymbella tumida</i> (Brébisson) Van Heurck	Intermediate	Biraphideae	5	1	0	0	1	0	0	HS5	16	4.44	6	1.55	0	0.00	19	4.74	0	0.00
<i>Cymbella turgidula</i> Grunow	Intermediate	Biraphideae	5	1	0	0	1	0	0	HS5	0	0.00	0	0.00	0	0.00	60	14.62	0	0.00
Σ-Cymbella sp.												4.44		2.20	0		37.42		2.33	
<i>Diploneis subovalis</i> Cleve	Pioneer	Biraphideae	4	1	0	0	0	1	0	LS4	0	0.00	0	0.00	0	0.00	0	0.00	18	4.26
<i>Diploneis zanzibarica</i> (Grunow) Hustedt	Pioneer	Biraphideae	4	1	0	0	0	1	0	LS4	0	0.00	6	1.47	0	0.00	0	0.00	0	0.00
Σ-Diploneis sp.												0		1.47	0		0		4.26	
<i>Discostella stelligera</i> (Cleve et Grun.) Houk & Klee	Pioneer	Centric	4	0	0	0	0	0	0	-	0	0.00	0	0.00	0	0.00	0	0.00	24	5.78
<i>Encyonema minutum</i> (Hilse in Rabh.) D.G. Mann	Late	Biraphideae	2	1	0	1	1	0	0	HS2	0	0.00	0	0.00	0	0.00	5	1.14	0	0.00
<i>Encyonema silesiacum</i> (Bleisch in Rabh.) D.G. Mann	Late	Biraphideae	4	1	0	1	1	0	0	HS4	0	0.00	0	0.00	0	0.00	0	0.00	1	0.16
Σ-Encyonema sp.												0		0	0		1.14		0.16	
<i>Eolimna minima</i> (Grunow) Lange-Bertalot	Intermediate	Biraphideae	1	1	0	0	0	0	1	MS1	0	0.00	0	0.00	0	0.00	11	2.70	0	0.00
<i>Epithemia sorex</i> Kützing	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	66	16.20	48	11.82	0	0.00	0	0.00
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	Pioneer	Araphideae	5	0	0	1	1	0	0	HS5	7	1.85	7	1.64	4	0.91	11	2.61	18	4.34
<i>Fragilaria ulna</i> (Nitzsch) Lange-Bertalot	Pioneer	Araphideae	5	0	0	1	1	0	0	HS5	4	1.20	0	0.00	0	0.00	0	0.00	0	0.00
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	Pioneer	Araphideae	3	0	0	1	1	0	0	HS3	0	0.00	0	0.00	0	0.00	12	2.86	18	4.42
<i>Fragilaria ungeriana</i> Grunow	Pioneer	Araphideae	3	0	0	1	1	0	0	HS3	0	0.00	0	0.00	0	0.00	2	0.49	0	0.00
Σ-Fragilaria sp.												3.05		1.64	0.91		5.96		8.76	
<i>Gomphonema aff. gracile</i>	Intermediate	Biraphideae	4	1	0	0	1	0	0	HS4	0	0.00	0	0.00	1	0.33	0	0.00	0	0.00
<i>Gomphonema affine</i> Kützing	Intermediate	Biraphideae	4	1	0	0	1	0	0	HS4	9	2.41	6	1.47	0	0.00	0	0.00	0	0.00
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	3	0.74	0	0.00	0	0.00	11	2.70	13	3.21
<i>Gomphonema exilissimum</i> Lange-Bertalot & Reichardt	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	2	0.46	0	0.00	0	0.00	0	0.00	0	0.00

<i>Gomphonema gracile</i> Ehrenberg	Intermediate	Biraphideae	4	1	0	0	1	0	0	HS4	0	0.00	0	0.00	3	0.74	0	0.00	0	0.00
<i>Gomphonema insigne</i> Gregory	Intermediate	Biraphideae	5	1	0	0	1	0	0	HS5	0	0.00	0	0.00	5	1.32	0	0.00	0	0.00
<i>Gomphonema lagenula</i> Kützing	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	0	0.00	0	0.00	2	0.50	0	0.00	0	0.00
<i>Gomphonema minutum</i> (Agardh) Agardh	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	39	10.83	11	2.78	5	1.24	0	0.00	0	0.00
<i>Gomphonema parvulus</i> Lange-Bertalot & Reichardt	Intermediate	Biraphideae	2	1	0	0	1	0	0	HS2	0	0.00	6	1.55	0	0.00	0	0.00	0	0.00
<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	0	0.00	0	0.00	3	0.66	4	0.98	2	0.48
<i>Gomphonema pseudoaugur</i> Lange-Bertalot	Intermediate	Biraphideae	4	1	0	0	1	0	0	HS4	0	0.00	4	0.90	4	0.91	0	0.00	0	0.00
<i>Gomphonema pumilum</i> (Grunow) Reichardt & Lange-Bertalot	Intermediate	Biraphideae	2	1	0	0	1	0	0	HS2	5	1.39	0	0.00	0	0.00	0	0.00	0	0.00
<i>Gomphonema venusta</i> Passy, Kociolek & Lowe	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	0	0.00	4	0.90	0	0.00	0	0.00	0	0.00
Σ-Gomphonema sp.												15.83		7.60		5.7		3.68		3.69
<i>Gyrosigma attenuatum</i> (Kützing) Rabenhorst	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	2	0.56	18	4.42	3	0.74	14	3.35	0	0.00
<i>Hantzschia distinctepunctata</i> Hustedt	Pioneer	Biraphideae	4	1	0	0	0	0	1	MS4	25	7.04	17	4.26	2	0.58	44	10.87	26	6.27
<i>Luticola mutica</i> (Kützing) DG Mann	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	0	0.00	0	0.00	3	0.74	0	0.00
<i>Mastogloia smithii</i> Thwaites	Pioneer	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	2	0.41	0	0.00	0	0.00	0	0.00
<i>Navicula cryptotenella</i> Lange-Bertalot	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	3	0.83	0	0.00	0	0.00	0	0.00	0	0.00
<i>Navicula radiosa</i> Kützing	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	0	0.00	16	4.05	8	1.96	39	9.40
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	31	8.61	6	1.47	21	5.21	5	1.14	4	1.04
<i>Navicula symmetrica</i> Patrick	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	7	1.80	11	2.64	0	0.00	0	0.00
<i>Navicula tripunctata</i> (O.F. Muller) Bory	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	7	1.94	0	0.00	11	2.73	0	0.00	0	0.00
<i>Navicula veneta</i> Kützing	Intermediate	Biraphideae	2	1	0	0	0	0	1	MS2	0	0.00	0	0.00	0	0.00	0	0.00	4	0.96
Σ-Navicula sp.												11.38		3.27		14.63		3.10		11.40
<i>Nitzschia closterium</i> (Ehrenberg) W Smith	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	0	0.00	0	0.00	0	0.00	9	2.09
<i>Nitzschia dissipata</i> (Kützing) Grunow	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	16	3.93	0	0.00	4	0.98	0	0.00

<i>Nitzschia dissipata</i> var. <i>media</i> (Hantzsch) Grunow	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	0	0.00	2	0.50	0	0.00	0	0.00
<i>Nitzschia frustulum</i> (Kützing) Grunow	Intermediate	Biraphideae	2	1	0	0	0	0	1	MS2	4	1.11	0	0.00	0	0.00	2	0.41	25	6.02
<i>Nitzschia lancettula</i> O Müller	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	3	0.93	0	0.00	0	0.00	0	0.00	0	0.00
<i>Nitzschia linearis</i> (Agardh) W Smith	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	0	0.00	0	0.00	0	0.00	13	3.21
<i>Nitzschia obtusa</i> var. <i>kurzii</i> Rabenhorst	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	5	1.23	0	0.00	1	0.33	0	0.00
<i>Nitzschia palea</i> (Kützing) W. Smith	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	32	8.80	0	0.00	42	10.50	10	2.45	27	6.59
<i>Nitzschia pumila</i> Hustedt	Intermediate	Biraphideae	2	1	0	0	0	0	0	-	0	0.00	0	0.00	0	0.00	0	0.00	14	3.37
<i>Nitzschia recta</i> Hantzsch	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	0	0.00	0	0.00	21	5.15	14	3.45
<i>Nitzschia reversa</i> W Smith	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	0	0.00	0	0.00	0	0.00	10	2.49
<i>Nitzschia sigma</i> (Kützing) W Smith	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	1	0.25	3	0.83	0	0.00	0	0.00
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	5	1.15	0	0.00	0	0.00	6	1.45
Σ-Nitzschia sp.												10.84		6.56		11.83		9.32		28.67
<i>Pinnularia borealis</i> Ehrenberg sensu lato	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	0	0.00	0	0.00	0	0.00	6	1.45
<i>Pinnularia divergens</i> W Smith	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	0	0.00	0	0.00	0	0.00	8	1.85
Σ-Pinnularia sp.												0		0		0		0		3.30
<i>Planothidium engelbrechtii</i> (Cholnoky) Round & Bukhityarova	Late	Monoraphideae	3	1	0	0	0	1	0	LS3	0	0.00	0	0.00	16	3.88	0	0.00	0	0.00
<i>Pleurosigma salinarum</i> Grunow	Pioneer	Biraphideae	5	1	0	0	0	0	0	-	0	0.00	0	0.00	0	0.00	0	0.00	28	6.83
<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-Bertalot	Late	Biraphideae	3	0	0	1	0	1	0	LS3	0	0.00	0	0.00	3	0.66	0	0.00	0	0.00
<i>Rhopalodia gibba</i> (Ehrenberg) O Müller	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	73	17.92	11	2.81	0	0.00	0	0.00
<i>Rhopalodia operculata</i> (Agardh) Håkansson	Pioneer	Biraphideae	3	1	0	0	0	0	1	MS3	0	0.00	0	0.00	8	1.90	3	0.65	0	0.00
Σ-Rhopalodia sp.												0		17.92		4.71		0.65		0
<i>Surirella brebissonii</i> Krammer & Lange-Bertalot	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	0	0.00	0	0.00	0	0.00	9	2.09
<i>Tryblionella apiculata</i> Gregory	Late	Biraphideae	4	1	0	0	0	0	1	MS4	7	1.85	3	0.65	2	0.41	0	0.00	6	1.45
<i>Tryblionella calida</i> (Grunow) DG Mann	Late	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	0	0.00	0	0.00	2	0.57	0	0.00

<i>Tryblionella gracilis</i> W Smith	Late	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	9	2.21	0	0.00	0	0.00	0	0.00
<i>Tryblionella littoralis</i> (Grunow) DG Mann	Late	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	0	0.00	0	0.00	0	0.00	6	1.37
<i>Σ-Tryblionella</i> sp.												1.85		2.86		0.41		0.57		2.82
% Diatom Community Characterisation											OLIF-1		OLIF-2		OLIF-3		OLIF-1*		OLIF-2*	
Colonisation strategy											Pioneer colonists		49.02		19.67		21.41		33.98	
											Intermediate colonists		44.03		71.16		64.95		57.19	
											Late colonists		6.96		9.17		13.64		8.84	
Group											Monoraphideae		23.81		41.16		6.21		5.78	
											Biraphideae		74.55		57.93		87.83		79.68	
											Araphideae		1.64		0.91		5.96		8.76	
											Centric		0.00		0.00		0.00		5.78	
Size class											Size 1		0.49		0.91		3.27		0.00	
											Size 2		5.65		2.40		9.48		12.93	
											Size 3		3.68		21.16		14.13		17.99	
											Size 4		16.37		14.05		19.44		28.19	
											Size 5		73.81		61.49		53.68		40.88	
Life-forms											Mobile		98.36		98.43		92.97		90.55	
											Pioneer		0.00		0.00		0.00		0.00	
											Colonial		1.64		1.57		7.03		9.45	
Ecological guilds											High profile		11.46		6.61		48.20		17.78	
											Low profile		28.56		44.46		8.58		14.34	
											Motile		59.98		48.93		43.22		67.88	
CEMFG's											HS1		0.00		0.00		0.00		0.00	
											HS2		1.36		0.00		1.14		0.00	
											HS3		3.21		2.12		7.03		9.66	
											HS4		2.07		1.75		0.00		0.19	
											HS5		17.48		12.40		40.03		7.93	
											LS1		0.43		0.80		0.57		0.00	
											LS2		0.00		0.00		0.00		0.00	
											LS3		0.00		4.01		0.00		0.00	
											LS4		0.00		1.31		0.00		5.07	
											LS5		23.18		34.43		8.01		9.27	
											MS1		0.00		0.00		2.70		0.00	
											MS2		3.57		2.12		8.33		11.38	
											MS3		0.00		12.55		7.11		11.76	
											MS4		10.91		10.65		19.44		21.41	
											MS5		37.80		17.87		5.64		23.33	

Appendix B3: List of diatom species sampled from the selected sites on the Limpopo River between April to May 2021.

Species	Colonisation strategy	Group	Size class	Life-forms			Ecological guilds			CEMFG's	Sites					
				Mobile	Pioneer	Colonial	High profile	Low profile	Motile		LIMP-1		LIMP-2		LIMP-3	
											µ-Count	%	µ-Count	%	µ-Count	%
<i>Achnanthyidium exiguum</i> (Grunow) Czamecki	Pioneer	Monoraphideae	1	1	0	0	0	1	0	LS1	18	4.19	0	0.00	0	0.00
<i>Amphora coffeaeformis</i> (Agardh) Kützing	Intermediate	Biraphideae	5	1	0	0	0	1	0	LS5	0	0.00	3	0.74	0	0.00
<i>Asterionella formosa</i> Hassal	Late	Araphideae	3	0	0	1	0	0	0	-	1	0.24	0	0.00	0	0.00
<i>Cocconeis placentula</i> Ehrenberg	Intermediate	Monoraphideae	5	1	0	0	0	1	0	LS5	3	0.71	26	6.36	16	3.86
<i>Craticula accomoda</i> (Hustedt) DG Mann	Late	Biraphideae	3	1	0	0	0	0	1	MS3	2	0.55	0	0.00	0	0.00
<i>Craticula ambigua</i> (Ehrenberg) DG Mann	Late	Biraphideae	3	1	0	0	0	0	1	MS3	6	1.42	0	0.00	0	0.00
<i>Craticula buderii</i> (Hustedt) Lange-Bertalot	Late	Biraphideae	2	1	0	0	0	0	1	MS2	69	16.28	39	9.66	23	5.74
<i>Craticula cuspidata</i> (Kützing) DG Mann	Late	Biraphideae	5	1	0	0	0	0	1	MS5	6	1.50	2	0.50	0	0.00
<i>Craticula halophila</i> (Grunow ex Van Heurck) Mann	Late	Biraphideae	3	1	0	0	0	0	1	MS3	0	0.00	4	0.99	57	13.95
Σ- <i>Craticula</i> sp.												19.75		11.15		19.69
<i>Cyclotella meneghiniana</i> Kützing	Pioneer	Centric	4	0	0	0	0	0	0	-	17	4.03	8	2.06	0	0.08
<i>Cymbella tumida</i> (Brébisson) Van Heurck	Intermediate	Biraphideae	5	1	0	0	1	0	0	HS5	0	0.00	4	0.99	0	0.00
<i>Diploneis elliptica</i> (Kützing) Cleve	Pioneer	Biraphideae	5	1	0	0	1	0	0	HS5	0	0.00	34	8.51	6	1.48
<i>Encyonema minutum</i> (Hilse in Rabh.) D.G. Mann	Late	Biraphideae	2	1	0	1	1	0	0	HS2	0	0.00	3	0.74	0	0.00
<i>Eolimna subminuscula</i> (Manguin) Lange-Bertalot	Intermediate	Biraphideae	2	1	0	0	0	0	1	MS2	2	0.47	0	0.00	3	0.74
<i>Fallacia monoculata</i> (Hustedt) DG Mann	Intermediate	Biraphideae	2	1	0	0	0	0	1	MS2	0	0.00	12	2.97	0	0.00
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	Pioneer	Araphideae	5	0	0	1	1	0	0	HS5	0	0.00	6	1.49	0	0.00
<i>Fragilaria ulna</i> (Nitzsch) Lange-Bertalot	Pioneer	Araphideae	5	0	0	1	1	0	0	HS5	0	0.00	11	2.64	0	0.00
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing) Lange-Bertalot	Pioneer	Araphideae	3	0	0	1	1	0	0	HS3	17	4.11	0	0.00	0	0.00
Σ- <i>Fragilaria</i> sp.												4.11		4.13		0
<i>Gomphonema affine</i> Kützing	Intermediate	Biraphideae	4	1	0	0	1	0	0	HS4	3	0.63	0	0.00	0	0.00
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	0	0.00	0	0.00	7	1.64
<i>Gomphonema lagenula</i> Kützing	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	6	1.34	0	0.00	0	0.00
<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	0	0.00	8	1.90	0	0.00
Σ- <i>Gomphonema</i> sp.												1.97		1.90		1.64
<i>Gyrosigma attenuatum</i> (Kützing) Rabenhorst	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	4	0.99	0	0.00
<i>Hantzschia distinctepunctata</i> Hustedt	Pioneer	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	5	1.24	0	0.00

<i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	Late	Biraphideae	3	1	0	0	0	0	1	MS3	0	0.00	1	0.25	0	0.00
<i>Luticola mutica</i> (Kützing) DG Mann	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	1	0.33	0	0.00
<i>Navicula antonii</i> Lange-Bertalot	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	21	4.98	0	0.00	0	0.00
<i>Navicula cryptotenella</i> Lange-Bertalot	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	1	0.24	53	13.21	0	0.00
<i>Navicula microcari</i> Lange-Bertalot	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	0	0.00	15	3.72	25	6.15
<i>Navicula radiosa</i> Kützing	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	29	7.27	31	7.55
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	23	5.45	0	0.00	15	3.69
<i>Navicula tripunctata</i> (OF Müller) Bory	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	61	14.55	0	0.00	9	2.30
Σ-Navicula sp.												25.22		24.20		19.69
<i>Nitzschia amphibia</i> Grunow	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	0	0.00	0	0.00	2	0.41
<i>Nitzschia closterium</i> (Ehrenberg) W Smith	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	1	0.24	0	0.00	0	0.00
<i>Nitzschia desertorum</i> Hustedt	Intermediate	Biraphideae	2	1	0	0	0	0	1	MS2	0	0.00	10	2.39	15	3.77
<i>Nitzschia dissipata</i> (Kützing) Grunow	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	17	4.21	7	1.72
<i>Nitzschia filiformis</i> (W Smith) Van Heurk	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	2	0.40	0	0.00	0	0.00
<i>Nitzschia frustulum</i> (Kützing) Grunow	Intermediate	Biraphideae	2	1	0	0	0	0	1	MS2	0	0.00	1	0.33	15	3.77
<i>Nitzschia littorea</i> Grunow	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	2	0.55	0	0.00	0	0.00
<i>Nitzschia obtusa</i> var. <i>kurzii</i> Rabenhorst	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	1	0.32	0	0.00	0	0.00
<i>Nitzschia palea</i> (Kützing) W. Smith	Intermediate	Biraphideae	3	1	0	0	0	0	1	MS3	22	5.14	0	0.00	31	7.71
<i>Nitzschia recta</i> Hantzsch	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	21	4.90	22	5.45	14	3.53
<i>Nitzschia reversa</i> W Smith	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	1	0.33	0	0.00
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	1	0.32	9	2.23	0	0.00
Σ-Nitzschia sp.												11.87		14.94		20.91
<i>Pinnularia</i> sp. Ehrenberg	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	2	0.58	0	0.00
<i>Pleurosigma salinarum</i> Grunow	Pioneer	Biraphideae	5	1	0	0	0	0	0	-	84	20.00	18	4.54	63	15.59
<i>Sellaphora pupula</i> (Kützing) Mereschkowsky sensu lato	Intermediate	Biraphideae	4	1	0	0	0	0	1	MS4	3	0.63	5	1.32	3	0.82
<i>Seminavis strigosa</i> (Hustedt) Danieleidis & Economou-Amilli	Intermediate	Biraphideae	3	1	0	0	0	1	0	LS3	0	0.00	13	3.22	0	0.00
<i>Simonsenia delognei</i> Lange-Bertalot	Intermediate	Biraphideae	1	1	0	0	0	0	1	MS1	1	0.32	0	0.00	0	0.00
<i>Stephanodiscus agassizensis</i> Håkansson & Kling	Pioneer	Centric	4	0	0	0	0	0	0	-	1	0.16	0	0.00	0	0.00
<i>Surirella angusta</i> Kützing	Pioneer	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	0	0.00	7	1.64
<i>Surirella brebissonii</i> Krammer & Lange-Bertalot	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	11	2.73	13	3.28
<i>Surirella ovalis</i> Brébisson	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	7	1.73	0	0.00
<i>Surirella tenera</i> Gregory	Pioneer	Biraphideae	5	1	0	0	0	0	1	MS5	0	0.00	5	1.16	2	0.57
Σ-Surirella sp.												0		5.62		5.49
<i>Tryblionella apiculata</i> Gregory	Late	Biraphideae	4	1	0	0	0	0	1	MS4	14	3.24	9	2.31	10	2.38
<i>Tryblionella coarctata</i> (Grunow) DG Mann	Late	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	4	0.91	4	0.98
<i>Tryblionella hungarica</i> (Grunow) DG Mann	Late	Biraphideae	4	1	0	0	0	0	1	MS4	6	1.34	0	0.00	0	0.00
<i>Tryblionella levidensis</i> W Smith	Late	Biraphideae	4	1	0	0	0	0	1	MS4	0	0.00	0	0.00	23	5.66
<i>Tryblionella littoralis</i> (Grunow) DG Mann	Late	Biraphideae	5	1	0	0	0	0	1	MS5	7	1.74	0	0.00	4	0.98
Σ-Tryblionella sp.												6.32		3.22		10

% Diatom Community Characterisation		LIMP-1	LIMP-2	LIMP-3
Colonisation strategy	Pioneer colonists	33.20	33.44	26.50
	Intermediate colonists	40.47	51.20	43.81
	Late colonists	26.32	15.36	29.70
Group	Monoraphideae	4.90	6.36	3.86
	Biraphideae	86.56	87.45	96.06
	Araphideae	4.35	4.13	0.00
	Centric	4.19	2.06	0.08
Size class	Size 1	4.51	0.00	0.00
	Size 2	16.76	16.10	14.03
	Size 3	18.58	23.29	29.86
	Size 4	35.34	18.74	22.81
	Size 5	24.82	41.87	33.31
Life-forms	Mobile	95.46	95.06	100.00
	Pioneer	0.00	0.00	0.00
	Colonial	4.54	4.94	0.00
Ecological guilds	High profile	8.05	17.42	3.70
	Low profile	6.49	11.05	4.57
	Motile	85.46	71.53	91.73
CEMFG's	HS1	0.00	0.00	0.00
	HS2	0.00	0.80	0.00
	HS3	7.22	2.03	1.95
	HS4	0.84	0.00	0.00
	HS5	0.00	14.59	1.75
	LS1	5.54	0.00	0.00
	LS2	0.00	0.00	0.00
	LS3	0.00	3.45	0.00
	LS4	0.00	0.00	0.00
	LS5	0.94	7.60	4.57
	MS1	0.42	0.00	0.00
	MS2	22.18	16.45	16.63
	MS3	17.05	19.45	33.46
	MS4	40.38	17.86	26.95
	MS5	5.44	17.77	14.69

Appendix B4: Contribution of various diatom species within group similarities determined through a Similarity percentage analysis (SIMPER). Groups are based on *T. granifera* density sampled at sites on the Phongolo River 2017, Olifants River 2020 and 2021 or Limpopo River 2021 and were determined using Bray Curtis similarity matrix.

<i>T. granifera</i> density	Overall similarity (%)	Species	Average abundance	Average similarity	Contribution (%)	Cumulative contribution (%)
Very high (>1500)	32.96	<i>Cocconeis placentula</i>	8.52	9.72	29.49	29.49
		<i>Gomphonema minutum</i>	4.77	4.64	14.09	43.58
		<i>Gomphonema affine</i>	3.88	3.18	9.66	53.24
		<i>Gyrosigma attenatum</i>	3.26	2.84	8.63	61.87
		<i>Hantzschia distinctepunctata</i>	3.07	1.58	4.8	66.66
		<i>Craticula buderi</i>	2.79	1.55	4.71	71.37
		<i>Nitzschia palea</i>	3.08	1.51	4.59	75.96
		<i>Amphora coffeaeformis</i>	3.09	1.39	4.21	80.17
		<i>Nitzschia dissipata</i>	2.33	1.25	3.78	83.95
		<i>Fragilaria biceps</i>	1.72	0.98	2.98	86.93
		<i>Cymbella tumida</i>	2.17	0.96	2.9	89.83
		<i>Navicula recens</i>	2.67	0.93	2.82	92.65
High (500-1500)	23.02	<i>Cocconeis placentula</i>	7.93	6.17	26.79	26.79
		<i>Nitzschia palea</i>	5.33	4.89	21.22	48.01
		<i>Tryblionella apiculata</i>	2.7	1.79	7.79	55.8
		<i>Navicula radiosa</i>	3.43	1.37	5.95	61.75
		<i>Nitzschia recta</i>	2.75	1.33	5.78	67.53
		<i>Craticula halophila</i>	2.69	1.3	5.65	73.18
		<i>Craticula buderi</i>	2.13	1.05	4.58	77.76
		<i>Amphora coffeaeformis</i>	1.6	0.71	3.06	80.82
		<i>Navicula recens</i>	2.22	0.71	3.06	83.89
		<i>Navicula veneta</i>	2.04	0.7	3.06	86.94
		<i>Nitzschia sigma</i>	2.06	0.7	3.03	89.97
		<i>Gyrosigma attenatum</i>	1.95	0.66	2.88	92.85
Medium (100-499)	43.92	<i>Cocconeis placentula</i>	10.13	14.46	32.93	32.93
		<i>Nitzschia</i> sp.	6.13	8.4	19.12	52.04
		<i>Navicula</i> sp.	6.12	7.82	17.8	69.84
		<i>Fragilaria ulna</i> var. <i>acus</i>	5.32	7.51	17.1	86.94
		<i>Gomphonema</i> sp.	4.22	5.74	13.06	100
Low (1-99)	29.79	<i>Craticula buderi</i>	6.74	5.91	19.85	19.85
		<i>Nitzschia recta</i>	4.61	4.59	15.4	35.24
		<i>Cocconeis placentula</i>	3.77	2.63	8.84	44.08
		<i>Pleurosigma salinarum</i>	4.49	1.45	4.88	48.96
		<i>Fragilaria ulna</i> var. <i>acus</i>	2.53	1.18	3.97	52.93
		<i>Nitzschia palea</i>	2.61	1.1	3.68	56.61
		<i>Tryblionella apiculata</i>	2.25	1.04	3.48	60.09
		<i>Cyclotella meneghiniana</i>	2.34	0.98	3.29	63.38

Appendix B4 continued.

<i>T. granifera</i> density	Overall similarity (%)	Species	Average abundance	Average similarity	Contribution (%)	Cumulative contribution (%)
		<i>Navicula radiosa</i>	2.75	0.91	3.04	66.42
		<i>Fragilaria biceps</i>	1.91	0.79	2.64	69.06
		<i>Navicula recens</i>	2.32	0.75	2.51	71.57
		<i>Hantzschia distinctepunctata</i>	2.96	0.72	2.41	73.98
		<i>Craticula halophila</i>	1.69	0.64	2.15	76.13
		<i>Cymbella tumida</i>	2.13	0.64	2.15	78.28
		<i>Gomphonema parvulum</i>	1.59	0.64	2.15	80.43
		<i>Gyrosigma attenatum</i>	1.9	0.64	2.15	82.59
		<i>Nitzschia dissipata</i>	2.04	0.64	2.15	84.74
		<i>Amphora coffeaeformis</i>	1.48	0.56	1.86	86.61
		<i>Encyonema minutum</i>	1.3	0.56	1.86	88.47
		<i>Sellaphora pupula</i>	1.31	0.55	1.86	90.33

Appendix B5: Contribution of various diatom species within pairwise group dissimilarities determined through a Similarity percentage analysis (SIMPER). Groups are based on *T. granifera* density sampled at sites on the Phongolo River 2017, Olifants River 2020 and 2021 or Limpopo River 2021 and were determined using Bray Curtis similarity matrix.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
			Medium	Very high			
		<i>Navicula</i> sp.	6.12	0	4.17	5.4	5.4
		<i>Gomphonema minutum</i>	0	4.77	3.24	4.2	9.6
		<i>Nitzschia</i> sp.	6.13	1.29	3.23	4.19	13.79
		<i>Aulacoseira granulata</i>	4.69	3.07	3.17	4.12	17.9
		<i>Gomphonema</i> sp.	4.22	0	2.86	3.71	21.61
		<i>Fragilaria ulna</i> var. <i>acus</i>	5.32	1.05	2.84	3.68	25.3
		<i>Gomphonema affine</i>	0	3.88	2.69	3.49	28.79
		<i>Navicula erifuga</i>	2.6	2.56	2.37	3.08	31.87
		<i>Gyrosigma attenatum</i>	0	3.26	2.23	2.9	34.76
		<i>Hantzschia distinctepunctata</i>	0	3.07	2.01	2.6	37.37
		<i>Navicula rostellata</i>	2.69	0	1.85	2.41	39.77
		<i>Rhopalodia gibba</i>	0	2.85	1.85	2.41	42.18
		<i>Craticula buderii</i>	0	2.79	1.82	2.37	44.54
		<i>Nitzschia siliqua</i>	2.65	0	1.82	2.36	46.91
		<i>Amphora coffeaeformis</i>	2.29	3.09	1.79	2.32	49.23
Medium & very high	77.09	<i>Epithemia sorex</i>	0	2.71	1.76	2.29	51.51
		<i>Navicula recens</i>	0	2.67	1.75	2.27	53.78
		<i>Nitzschia palea</i>	2.12	3.08	1.75	2.27	56.05
		<i>Fragilaria tenera</i>	2.5	0	1.72	2.23	58.29
		<i>Nitzschia dissipata</i>	0	2.33	1.6	2.07	60.36
		<i>Navicula radiosa</i>	2.35	0	1.57	2.04	62.39
		<i>Tryblionella apiculata</i>	2.29	1.41	1.57	2.03	64.42
		<i>Cyclotella meneghiniana</i>	2.24	0	1.5	1.94	66.36
		<i>Achnantheidium</i> sp.	2.12	0	1.46	1.9	68.26
		<i>Cymbella tumida</i>	0	2.17	1.42	1.84	70.1
		<i>Gomphonema parvulum</i>	1.73	0	1.16	1.5	71.61
		<i>Gyrosigma</i> sp.	1.73	0	1.16	1.5	73.11
		<i>Fragilaria biceps</i>	0	1.72	1.13	1.46	74.57
		<i>Cocconeis placentula</i>	10.13	8.52	1.12	1.45	76.02
		<i>Cocconeis pediculus</i>	0	1.67	1.09	1.42	77.44
		<i>Rhopalodia gibberula</i>	0	1.49	1.09	1.41	78.85

Appendix B5 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Mastogloia smithii</i>	0	1.37	0.97	1.25	80.11
		<i>Encyonema silesiacum</i>	0	1.29	0.94	1.22	81.33
		<i>Navicula veneta</i>	0	1.29	0.94	1.22	82.55
		<i>Capartogramma crucicula</i>	1.32	0	0.88	1.15	83.7
		<i>Seminavis strigosa</i>	0	1.2	0.88	1.14	84.83
		<i>Achnantheidium exiguum</i>	0	1.33	0.87	1.13	85.96
		<i>Tryblionella gracilis</i>	0	1	0.65	0.84	86.81
		<i>Amphora fontinalis</i>	0	0.94	0.62	0.8	87.61
		<i>Navicula symmetrica</i>	0	0.9	0.59	0.76	88.37
		<i>Navicula tripunctata</i>	0	0.88	0.58	0.75	89.12
		<i>Amphora veneta</i>	0	0.84	0.55	0.71	89.84
		<i>Gomphonema parvulus</i>	0	0.84	0.55	0.71	90.55
			Medium	High			
		<i>Aulacoseira granulata</i>	4.69	0	2.93	4.03	4.03
		<i>Nitzschia</i> sp.	6.13	1.67	2.76	3.81	7.84
		<i>Navicula</i> sp.	6.12	1.91	2.74	3.77	11.6
		<i>Fragilaria ulna</i> var. <i>acus</i>	5.32	1.43	2.53	3.48	15.09
		<i>Nitzschia palea</i>	2.12	5.33	2.04	2.8	17.89
		<i>Cocconeis placentula</i>	10.13	7.93	1.98	2.72	20.61
		<i>Gomphonema</i> sp.	4.22	1.6	1.85	2.55	23.16
		<i>Navicula radiosa</i>	2.35	3.43	1.79	2.46	25.63
		<i>Nitzschia recta</i>	0	2.75	1.74	2.4	28.02
		<i>Navicula rostellata</i>	2.69	0	1.73	2.38	30.4
		<i>Craticula halophila</i>	0	2.69	1.7	2.34	32.74
		<i>Nitzschia siliqua</i>	2.65	0	1.7	2.34	35.08
		<i>Navicula erifuga</i>	2.6	1.45	1.65	2.28	37.35
		<i>Fragilaria tenera</i>	2.5	0	1.6	2.21	39.56
		<i>Epithemia sorex</i>	0	2.3	1.48	2.03	41.59
		<i>Tryblionella apiculata</i>	2.29	2.7	1.45	1.99	43.58
		<i>Amphora coffeaeformis</i>	2.29	1.6	1.44	1.99	45.57
		<i>Cyclotella meneghiniana</i>	2.24	1.45	1.41	1.94	47.51
		<i>Navicula recens</i>	0	2.22	1.38	1.91	49.42
		<i>Nitzschia sigma</i>	0	2.06	1.37	1.88	51.3
		<i>Achnantheidium</i> sp.	2.12	0	1.36	1.87	53.17
		<i>Hantzschia distinctepunctata</i>	0	2.21	1.32	1.82	54.99
		<i>Navicula veneta</i>	0	2.04	1.31	1.81	56.8
		<i>Craticula buderi</i>	0	2.13	1.3	1.79	58.59
		<i>Gyrosigma attenuatum</i>	0	1.95	1.29	1.78	60.37
		<i>Fragilaria biceps</i>	0	2.05	1.24	1.7	62.07
		<i>Nitzschia dissipata</i>	0	1.7	1.14	1.57	63.65
		<i>Tryblionella levidensis</i>	0	1.63	1.1	1.51	65.16
		<i>Gomphonema parvulum</i>	1.73	1.02	1.09	1.5	66.66
		<i>Gyrosigma</i> sp.	1.73	0	1.08	1.49	68.15
		<i>Nitzschia filiformis</i>	0	1.56	1.05	1.45	69.6
		<i>Pleurosigma salinarum</i>	0	1.77	1.04	1.43	71.02
		<i>Nitzschia frustulum</i>	0	1.67	0.97	1.34	72.37
		<i>Discostella stelligera</i>	0	1.63	0.95	1.31	73.68
		<i>Hippodonta</i> sp.	0	1.29	0.87	1.2	74.87
		<i>Planothidium engelbrechtii</i>	0	1.32	0.85	1.17	76.04
		<i>Capartogramma crucicula</i>	1.32	0	0.83	1.14	77.18
		<i>Diploneis subovalis</i>	0	1.4	0.82	1.13	78.3
		<i>Eolimna subminuscula</i>	0	1.15	0.78	1.07	79.37
		<i>Cocconeis pediculus</i>	0	1.14	0.73	1.01	80.38
		<i>Nitzschia pumila</i>	0	1.25	0.73	1	81.38
		<i>Rhopalodia gibba</i>	0	1.12	0.72	0.99	82.37
		<i>Gomphonema agustatum</i>	0	1.22	0.71	0.98	83.35
		<i>Nitzschia linearis</i>	0	1.22	0.71	0.98	84.33
		<i>Navicula tripunctata</i>	0	1.11	0.71	0.98	85.31
		<i>Navicula symmetrica</i>	0	1.09	0.7	0.96	86.27
		<i>Nitzschia reversa</i>	0	1.07	0.63	0.86	87.13
		<i>Cymbella aspera</i>	0	1.04	0.61	0.83	87.97
Medium & high	72.59						

Appendix B5 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Rhopalodia operculata</i>	0	0.92	0.59	0.82	88.78
		<i>Caloneis hyalina</i>	0	0.9	0.58	0.8	89.58
		<i>Nitzschia closterium</i>	0	0.98	0.57	0.79	90.37
			Very high	High			
		<i>Gomphonema minutum</i>	4.77	0.75	2.29	3.28	3.28
		<i>Gomphonema affine</i>	3.88	0	2.26	3.23	6.51
		<i>Epithemia sorex</i>	2.71	2.3	1.97	2.82	9.33
		<i>Navicula radiosa</i>	0	3.43	1.89	2.7	12.02
		<i>Aulacoseira granulata</i>	3.07	0	1.86	2.67	14.69
		<i>Navicula erifuga</i>	2.56	1.45	1.81	2.58	17.27
		<i>Rhopalodia gibba</i>	2.85	1.12	1.8	2.58	19.85
		<i>Nitzschia recta</i>	0	2.75	1.57	2.25	22.1
		<i>Nitzschia palea</i>	3.08	5.33	1.49	2.13	24.23
		<i>Cocconeis placentula</i>	8.52	7.93	1.48	2.12	26.35
		<i>Hantzschia distinctepunctata</i>	3.07	2.21	1.46	2.09	28.44
		<i>Amphora coffeaeformis</i>	3.09	1.6	1.45	2.08	30.52
		<i>Craticula halophila</i>	0.61	2.69	1.42	2.03	32.56
		<i>Nitzschia dissipata</i>	2.33	1.7	1.42	2.03	34.59
		<i>Navicula recens</i>	2.67	2.22	1.38	1.97	36.56
		<i>Nitzschia</i> sp.	1.29	1.67	1.24	1.77	38.33
		<i>Cymbella tumida</i>	2.17	0	1.2	1.72	40.05
		<i>Craticula buderii</i>	2.79	2.13	1.2	1.71	41.77
		<i>Navicula veneta</i>	1.29	2.04	1.16	1.66	43.43
		<i>Nitzschia sigma</i>	0.33	2.06	1.16	1.66	45.09
		<i>Navicula</i> sp.	0	1.91	1.16	1.65	46.74
		<i>Gyrosigma attenuatum</i>	3.26	1.95	1.1	1.58	48.32
		<i>Nitzschia frustulum</i>	0.67	1.67	1.03	1.47	49.79
		<i>Fragilaria biceps</i>	1.72	2.05	1	1.44	51.23
		<i>Fragilaria ulna</i> var. <i>acus</i>	1.05	1.43	1	1.43	52.66
		<i>Tryblionella apiculata</i>	1.41	2.7	1	1.43	54.09
		<i>Tryblionella levidensis</i>	0	1.63	0.99	1.41	55.5
		<i>Gomphonema</i> sp.	0	1.6	0.96	1.38	56.88
		<i>Cocconeis pediculus</i>	1.67	1.14	0.96	1.37	58.26
		<i>Nitzschia filiformis</i>	0	1.56	0.94	1.35	59.61
		<i>Pleurosigma salinarum</i>	0	1.77	0.94	1.35	60.96
		<i>Rhopalodia gibberula</i>	1.49	0	0.9	1.29	62.25
		<i>Cyclotella meneghiniana</i>	0	1.45	0.88	1.25	63.5
		<i>Discostella stelligera</i>	0	1.63	0.87	1.24	64.75
		<i>Encyonema silesiacum</i>	1.29	0.27	0.83	1.18	65.93
		<i>Mastogloia smithii</i>	1.37	0	0.81	1.16	67.08
		<i>Navicula tripunctata</i>	0.88	1.11	0.8	1.14	68.23
		<i>Navicula symmetrica</i>	0.9	1.09	0.79	1.13	69.36
		<i>Hippodonta</i> sp.	0	1.29	0.78	1.11	70.47
		<i>Planothidium engelbrechtii</i>	0	1.32	0.76	1.09	71.57
		<i>Gomphonema agustatum</i>	0.54	1.22	0.76	1.09	72.65
		<i>Diploneis subovalis</i>	0	1.4	0.74	1.07	73.72
		<i>Achnantheidium exiguum</i>	1.33	0	0.74	1.06	74.78
		<i>Seminavis strigosa</i>	1.2	0	0.73	1.04	75.82
		<i>Eolimna subminuscula</i>	0	1.15	0.7	1	76.82
		<i>Cymbella aspera</i>	0.54	1.04	0.66	0.95	77.77
		<i>Nitzschia pumila</i>	0	1.25	0.66	0.95	78.71
		<i>Nitzschia linearis</i>	0	1.22	0.65	0.93	79.64
		<i>Nitzschia umbonata</i>	0.72	0.82	0.58	0.84	80.48
		<i>Nitzschia reversa</i>	0	1.07	0.57	0.82	81.29
		<i>Gomphonema parvulum</i>	0	1.02	0.57	0.81	82.1
		<i>Amphora veneta</i>	0.84	0.47	0.56	0.8	82.9
		<i>Tryblionella gracilis</i>	1	0	0.55	0.79	83.69
		<i>Rhopalodia operculata</i>	0	0.92	0.53	0.76	84.46
		<i>Amphora fontinalis</i>	0.94	0	0.52	0.75	85.21
		<i>Caloneis hyalina</i>	0	0.9	0.52	0.75	85.95
		<i>Amphora pediculus</i>	0.67	0.69	0.52	0.75	86.7
Very high & high	69.89						

Appendix B5 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Nitzschia closterium</i>	0	0.98	0.52	0.75	87.45
		<i>Surirella brebissoni</i>	0	0.98	0.52	0.75	88.19
		<i>Pinnularia divergens</i>	0	0.92	0.49	0.7	88.89
		<i>Gomphonema pseudoaugur</i>	0.64	0.64	0.48	0.69	89.59
		<i>Gomphonema parvulus</i>	0.84	0	0.46	0.66	90.25
			Medium	Low			
		<i>Craticula buderi</i>	0	6.74	4.12	4.87	4.87
		<i>Cocconeis placentula</i>	10.13	3.77	3.91	4.61	9.48
		<i>Nitzschia</i> sp.	6.13	0	3.73	4.41	13.88
		<i>Navicula</i> sp.	6.12	0	3.73	4.4	18.28
		<i>Aulacoseira granulata</i>	4.69	0	2.82	3.32	21.61
		<i>Nitzschia recta</i>	0	4.61	2.8	3.3	24.91
		<i>Pleurosigma salinarum</i>	0	4.49	2.8	3.3	28.22
		<i>Gomphonema</i> sp.	4.22	0	2.56	3.02	31.24
		<i>Hantzschia distinctepunctata</i>	0	2.96	1.76	2.08	33.32
		<i>Navicula tripunctata</i>	0	2.61	1.68	1.98	35.3
		<i>Fragilaria ulna</i> var. <i>acus</i>	5.32	2.53	1.66	1.96	37.26
		<i>Navicula rostellata</i>	2.69	0	1.66	1.96	39.21
		<i>Navicula cryptotenella</i>	0	2.77	1.64	1.93	41.15
		<i>Nitzschia siliqua</i>	2.65	0	1.63	1.92	43.07
		<i>Navicula erifuga</i>	2.6	0	1.6	1.89	44.96
		<i>Navicula radiosa</i>	2.35	2.75	1.57	1.85	46.81
		<i>Fragilaria tenera</i>	2.5	0	1.54	1.82	48.63
		<i>Cymbella turgidula</i>	0	2.57	1.54	1.82	50.44
		<i>Navicula recens</i>	0	2.32	1.46	1.72	52.16
		<i>Tryblionella apiculata</i>	2.29	2.25	1.39	1.64	53.81
		<i>Amphora coffeaeformis</i>	2.29	1.48	1.39	1.64	55.44
		<i>Nitzschia palea</i>	2.12	2.61	1.38	1.63	57.07
		<i>Cyclotella meneghiniana</i>	2.24	2.34	1.36	1.61	58.68
		<i>Achnanthydium</i> sp.	2.12	0	1.31	1.54	60.22
		<i>Cymbella tumida</i>	0	2.13	1.27	1.49	61.72
		<i>Cymbella kolbei</i>	0	2.04	1.22	1.44	63.16
Medium & low	84.74	<i>Achnanthydium eutrophilum</i>	0	1.91	1.2	1.42	64.58
		<i>Nitzschia dissipata</i>	0	2.04	1.2	1.42	66
		<i>Diploneis elliptica</i>	0	1.95	1.14	1.35	67.34
		<i>Fragilaria biceps</i>	0	1.91	1.13	1.33	68.67
		<i>Gyrosigma attenuatum</i>	0	1.9	1.13	1.33	70
		<i>Gomphonema parvulum</i>	1.73	1.59	1.05	1.24	71.24
		<i>Gyrosigma</i> sp.	1.73	0	1.04	1.23	72.47
		<i>Craticula halophila</i>	0	1.69	1	1.18	73.65
		<i>Navicula antonii</i>	0	1.53	0.98	1.16	74.81
		<i>Cymbella neocistula</i>	0	1.54	0.92	1.09	75.89
		<i>Nitzschia umbonata</i>	0	1.38	0.83	0.98	76.88
		<i>Craticula cuspidata</i>	0	1.31	0.81	0.96	77.84
		<i>Sellaphora pupula</i>	0	1.31	0.8	0.94	78.78
		<i>Capartogramma crucicula</i>	1.32	0	0.79	0.94	79.72
		<i>Cymbella aspera</i>	0	1.29	0.77	0.91	80.63
		<i>Encyonema minutum</i>	0	1.3	0.77	0.91	81.53
		<i>Navicula microcari</i>	0	1.29	0.75	0.89	82.42
		<i>Seminavis strigosa</i>	0	1.2	0.7	0.83	83.25
		<i>Fallacia monoculata</i>	0	1.15	0.67	0.8	84.05
		<i>Eolimna minima</i>	0	1.11	0.66	0.78	84.83
		<i>Gomphonema agustatum</i>	0	1.11	0.66	0.78	85.61
		<i>Surirella brebissoni</i>	0	1.11	0.65	0.76	86.37
		<i>Fragilaria ulna</i>	0	1.09	0.64	0.75	87.12
		<i>Nitzschia desertorum</i>	0	1.04	0.61	0.71	87.84
		<i>Tryblionella littoralis</i>	0	0.9	0.58	0.68	88.52
		<i>Luticola mutica</i>	0	0.96	0.57	0.67	89.19
		<i>Caloneis molaris</i>	0	0.88	0.53	0.62	89.82
		<i>Craticula ambigua</i>	0	0.82	0.52	0.62	90.44

Appendix B5 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
			Very high	Low			
Very high & low	75.51	<i>Cocconeis placentula</i>	8.52	3.77	2.63	3.49	3.49
		<i>Gomphonema minutum</i>	4.77	0	2.63	3.48	6.97
		<i>Nitzschia recta</i>	0	4.61	2.54	3.36	10.33
		<i>Pleurosigma salinarum</i>	0	4.49	2.53	3.35	13.68
		<i>Craticula buderii</i>	2.79	6.74	2.24	2.97	16.65
		<i>Gomphonema affine</i>	3.88	0.54	1.86	2.47	19.12
		<i>Aulacoseira granulata</i>	3.07	0	1.79	2.38	21.5
		<i>Navicula tripunctata</i>	0.88	2.61	1.65	2.19	23.69
		<i>Hantzschia distinctepunctata</i>	3.07	2.96	1.65	2.19	25.87
		<i>Rhopalodia gibba</i>	2.85	0	1.52	2.01	27.88
		<i>Navicula erifuga</i>	2.56	0	1.5	1.98	29.87
		<i>Navicula cryptotenella</i>	0.58	2.77	1.47	1.95	31.82
		<i>Navicula radiosa</i>	0	2.75	1.47	1.95	33.76
		<i>Epithemia sorex</i>	2.71	0	1.44	1.91	35.68
		<i>Amphora coffeaeformis</i>	3.09	1.48	1.42	1.88	37.56
		<i>Cymbella turgidula</i>	0	2.57	1.4	1.85	39.41
		<i>Navicula recens</i>	2.67	2.32	1.34	1.77	41.18
		<i>Nitzschia palea</i>	3.08	2.61	1.33	1.76	42.94
		<i>Cyclotella meneghiniana</i>	0	2.34	1.31	1.73	44.67
		<i>Fragilaria ulna</i> var. <i>acus</i>	1.05	2.53	1.2	1.59	46.26
		<i>Gyrosigma attenuatum</i>	3.26	1.9	1.12	1.49	47.75
		<i>Cymbella kolbei</i>	0	2.04	1.11	1.46	49.21
		<i>Cymbella tumida</i>	2.17	2.13	1.09	1.44	50.66
		<i>Achnanthydium eutrophilum</i>	0	1.91	1.09	1.44	52.1
		<i>Nitzschia dissipata</i>	2.33	2.04	1.07	1.41	53.51
		<i>Diploneis elliptica</i>	0	1.95	1.04	1.38	54.88
		<i>Tryblionella apiculata</i>	1.41	2.25	1	1.32	56.2
		<i>Seminavis strigosa</i>	1.2	1.2	0.89	1.18	57.38
		<i>Navicula antonii</i>	0	1.53	0.88	1.17	58.56
		<i>Rhopalodia gibberula</i>	1.49	0	0.87	1.15	59.71
		<i>Gomphonema parvulum</i>	0	1.59	0.85	1.13	60.84
		<i>Cymbella neocistula</i>	0	1.54	0.84	1.11	61.95
		<i>Craticula halophila</i>	0.61	1.69	0.81	1.07	63.02
		<i>Cocconeis pediculus</i>	1.67	0.54	0.81	1.07	64.09
		<i>Cymbella aspera</i>	0.54	1.29	0.8	1.06	65.15
		<i>Mastogloia smithii</i>	1.37	0	0.78	1.03	66.18
		<i>Fragilaria biceps</i>	1.72	1.91	0.78	1.03	67.21
		<i>Encyonema silesiacum</i>	1.29	0	0.75	1	68.21
		<i>Navicula veneta</i>	1.29	0	0.75	1	69.21
		<i>Nitzschia</i> sp.	1.29	0	0.75	1	70.21
		<i>Nitzschia umbonata</i>	0.72	1.38	0.75	0.99	71.2
		<i>Craticula cuspidata</i>	0	1.31	0.74	0.98	72.17
		<i>Sellaphora pupula</i>	0	1.31	0.72	0.96	73.13
		<i>Achnanthydium exiguum</i>	1.33	0	0.71	0.94	74.08
		<i>Fragilaria ulna</i>	0.69	1.09	0.71	0.94	75.02
		<i>Gomphonema agustatum</i>	0.54	1.11	0.7	0.93	75.95
		<i>Encyonema minutum</i>	0	1.3	0.7	0.92	76.87
		<i>Navicula microcari</i>	0	1.29	0.69	0.91	77.78
		<i>Fallacia monoculata</i>	0	1.15	0.61	0.81	78.59
		<i>Eolimna minima</i>	0	1.11	0.6	0.8	79.39
		<i>Surirella brebissoni</i>	0	1.11	0.59	0.78	80.17
		<i>Nitzschia desertorum</i>	0	1.04	0.55	0.73	80.9
		<i>Nitzschia obtusa</i>	0.75	0.77	0.55	0.73	81.63
		<i>Tryblionella gracilis</i>	1	0	0.53	0.71	82.33
		<i>Amphora veneta</i>	0.84	0.38	0.52	0.69	83.03
		<i>Tryblionella littoralis</i>	0	0.9	0.52	0.69	83.72
		<i>Luticola mutica</i>	0	0.96	0.52	0.69	84.4
		<i>Nitzschia frustulum</i>	0.67	0.82	0.51	0.68	85.08
		<i>Amphora fontinalis</i>	0.94	0	0.51	0.67	85.75
		<i>Navicula symmetrica</i>	0.9	0	0.48	0.64	86.39

Appendix B5 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Caloneis molaris</i>	0	0.88	0.48	0.63	87.02
		<i>Craticula ambigua</i>	0	0.82	0.47	0.63	87.65
		<i>Surirella ovalis</i>	0	0.88	0.47	0.62	88.27
		<i>Gomphonema lagenula</i>	0	0.79	0.46	0.61	88.88
		<i>Tryblionella hungarica</i>	0	0.79	0.46	0.61	89.49
		<i>Gomphonema parvulus</i>	0.84	0	0.45	0.59	90.08
			High	Low			
		<i>Craticula buderi</i>	2.13	6.74	2.45	3.48	3.48
		<i>Cocconeis placentula</i>	7.93	3.77	2.24	3.19	6.67
		<i>Pleurosigma salinarum</i>	1.77	4.49	2.2	3.13	9.8
		<i>Navicula tripunctata</i>	1.11	2.61	1.6	2.27	12.08
		<i>Hantzschia distinctepunctata</i>	2.21	2.96	1.48	2.11	14.18
		<i>Navicula radiosa</i>	3.43	2.75	1.46	2.08	16.26
		<i>Nitzschia palea</i>	5.33	2.61	1.43	2.04	18.3
		<i>Navicula cryptotenella</i>	0	2.77	1.41	2	20.3
		<i>Cymbella turgidula</i>	0	2.57	1.32	1.88	22.17
		<i>Nitzschia dissipata</i>	1.7	2.04	1.24	1.76	23.94
		<i>Epithemia sorex</i>	2.3	0	1.21	1.72	25.66
		<i>Fragilaria ulna</i> var. <i>acus</i>	1.43	2.53	1.2	1.71	27.37
		<i>Cyclotella meneghiniana</i>	1.45	2.34	1.16	1.65	29.02
		<i>Craticula halophila</i>	2.69	1.69	1.12	1.59	30.61
		<i>Nitzschia sigma</i>	2.06	0	1.11	1.59	32.19
		<i>Navicula recens</i>	2.22	2.32	1.11	1.58	33.78
		<i>Cymbella tumida</i>	0	2.13	1.09	1.55	35.33
		<i>Navicula veneta</i>	2.04	0	1.08	1.53	36.86
		<i>Navicula</i> sp.	1.91	0	1.05	1.49	38.35
		<i>Cymbella kolbei</i>	0	2.04	1.04	1.49	39.83
		<i>Diploneis elliptica</i>	0	1.95	0.98	1.4	41.23
		<i>Nitzschia frustulum</i>	1.67	0.82	0.97	1.38	42.61
		<i>Nitzschia recta</i>	2.75	4.61	0.96	1.37	43.98
		<i>Fragilaria biceps</i>	2.05	1.91	0.95	1.35	45.33
		<i>Gyrosigma attenuatum</i>	1.95	1.9	0.95	1.35	46.68
		<i>Achnanthyidium eutrophilum</i>	0.64	1.91	0.95	1.35	48.03
		<i>Tryblionella apiculata</i>	2.7	2.25	0.93	1.33	49.36
		<i>Nitzschia filiformis</i>	1.56	0.43	0.92	1.32	50.68
		<i>Nitzschia</i> sp.	1.67	0	0.91	1.3	51.97
		<i>Tryblionella levidensis</i>	1.63	0	0.89	1.27	53.25
		<i>Gomphonema</i> sp.	1.6	0	0.87	1.24	54.49
		<i>Cymbella aspera</i>	1.04	1.29	0.83	1.19	55.68
		<i>Navicula antonii</i>	0	1.53	0.83	1.18	56.86
		<i>Gomphonema agustatum</i>	1.22	1.11	0.81	1.15	58.01
		<i>Discostella stelligera</i>	1.63	0	0.8	1.13	59.14
		<i>Navicula erifuga</i>	1.45	0	0.79	1.13	60.27
		<i>Cymbella neocistula</i>	0	1.54	0.79	1.12	61.39
		<i>Surirella brebissoni</i>	0.98	1.11	0.73	1.03	62.42
		<i>Nitzschia umbonata</i>	0.82	1.38	0.72	1.03	63.45
		<i>Eolimna subminuscula</i>	1.15	0.47	0.71	1.01	64.46
		<i>Hippodonta</i> sp.	1.29	0	0.71	1	65.46
		<i>Planothidium engelbrechtii</i>	1.32	0	0.69	0.99	66.45
		<i>Craticula cuspidata</i>	0	1.31	0.69	0.99	67.44
		<i>Cocconeis pediculus</i>	1.14	0.54	0.69	0.98	68.42
		<i>Sellaphora pupula</i>	0	1.31	0.68	0.97	69.39
		<i>Diploneis subovalis</i>	1.4	0	0.68	0.97	70.37
		<i>Encyonema minutum</i>	0	1.3	0.66	0.94	71.3
		<i>Amphora coffeaeformis</i>	1.6	1.48	0.65	0.93	72.23
		<i>Gomphonema parvulum</i>	1.02	1.59	0.65	0.92	73.15
		<i>Navicula microcari</i>	0	1.29	0.65	0.92	74.08
		<i>Tryblionella littoralis</i>	0.79	0.9	0.61	0.87	74.94
		<i>Nitzschia pumila</i>	1.25	0	0.61	0.86	75.81
		<i>Seminavis strigosa</i>	0	1.2	0.6	0.86	76.67
		<i>Nitzschia reversa</i>	1.07	0.38	0.59	0.85	77.51
High & low	70.29						

Appendix B5 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Nitzschia linearis</i>	1.22	0	0.59	0.84	78.36
		<i>Rhopalodia gibba</i>	1.12	0	0.59	0.84	79.2
		<i>Fallacia monoculata</i>	0	1.15	0.58	0.83	80.02
		<i>Rhopalodia operculata</i>	0.92	0.54	0.58	0.82	80.84
		<i>Navicula symmetrica</i>	1.09	0	0.57	0.81	81.66
		<i>Eolimna minima</i>	0	1.11	0.57	0.81	82.47
		<i>Fragilaria ulna</i>	0	1.09	0.55	0.78	83.24
		<i>Nitzschia closterium</i>	0.98	0.33	0.55	0.78	84.02
		<i>Nitzschia desertorum</i>	0	1.04	0.52	0.74	84.76
		<i>Gomphonema lagenula</i>	0.47	0.79	0.51	0.72	85.48
		<i>Luticola mutica</i>	0	0.96	0.49	0.7	86.18
		<i>Caloneis hyalina</i>	0.9	0	0.48	0.68	86.86
		<i>Caloneis molaris</i>	0	0.88	0.45	0.64	87.5
		<i>Pinnularia divergens</i>	0.92	0	0.45	0.64	88.14
		<i>Craticula ambigua</i>	0	0.82	0.44	0.63	88.77
		<i>Surirella ovalis</i>	0	0.88	0.44	0.63	89.4
		<i>Tryblionella hungarica</i>	0	0.79	0.43	0.62	90.02
			Medium	None			
		<i>Pleurosigma salinarum</i>	0	7.96	5.07	5.83	5.83
		<i>Craticula halophila</i>	0	7.53	4.8	5.52	11.35
		<i>Cocconeis placentula</i>	10.13	3.96	3.94	4.53	15.87
		<i>Nitzschia</i> sp.	6.13	0	3.91	4.5	20.37
		<i>Navicula</i> sp.	6.12	0	3.91	4.49	24.86
		<i>Fragilaria ulna</i> var. <i>acus</i>	5.32	0	3.39	3.9	28.76
		<i>Navicula microcari</i>	0	5	3.19	3.66	32.42
		<i>Craticula buderii</i>	0	4.83	3.08	3.54	35.96
		<i>Tryblionella levidensis</i>	0	4.8	3.06	3.51	39.48
		<i>Aulacoseira granulata</i>	4.69	0	2.95	3.39	42.87
		<i>Gomphonema</i> sp.	4.22	0	2.68	3.08	45.95
		<i>Nitzschia desertorum</i>	0	3.92	2.5	2.87	48.82
		<i>Nitzschia frustulum</i>	0	3.92	2.5	2.87	51.69
		<i>Navicula recens</i>	0	3.87	2.47	2.84	54.53
		<i>Nitzschia recta</i>	0	3.79	2.41	2.77	57.3
		<i>Surirella brebissoni</i>	0	3.65	2.33	2.68	59.98
		<i>Nitzschia palea</i>	2.12	5.6	2.23	2.57	62.54
		<i>Navicula radiosa</i>	2.35	5.54	2.05	2.36	64.91
		<i>Navicula tripunctata</i>	0	3.06	1.95	2.24	67.15
		<i>Navicula rostellata</i>	2.69	0	1.74	2	69.15
		<i>Nitzschia siliqua</i>	2.65	0	1.71	1.97	71.11
		<i>Nitzschia dissipata</i>	0	2.65	1.69	1.94	73.05
		<i>Navicula erifuga</i>	2.6	0	1.68	1.93	74.98
		<i>Gomphonema agustatum</i>	0	2.58	1.65	1.89	76.87
		<i>Surirella angusta</i>	0	2.58	1.65	1.89	78.76
		<i>Fragilaria tenera</i>	2.5	0	1.61	1.86	80.62
		<i>Diploneis elliptica</i>	0	2.45	1.56	1.79	82.41
		<i>Tryblionella apiculata</i>	2.29	3.11	1.45	1.67	84.08
		<i>Amphora coffeaeformis</i>	2.29	0	1.44	1.66	85.74
		<i>Cyclotella meneghiniana</i>	2.24	0.58	1.41	1.62	87.36
		<i>Achnanthydium</i> sp.	2.12	0	1.37	1.58	88.94
		<i>Tryblionella coarctata</i>	0	2	1.27	1.47	90.4
			Very high	None			
		<i>Pleurosigma salinarum</i>	0	7.96	4.58	5.74	5.74
		<i>Craticula halophila</i>	0.61	7.53	3.99	5	10.74
		<i>Navicula radiosa</i>	0	5.54	3.18	3.99	14.73
		<i>Navicula microcari</i>	0	5	2.88	3.6	18.33
		<i>Tryblionella levidensis</i>	0	4.8	2.76	3.46	21.79
		<i>Gomphonema minutum</i>	4.77	0	2.74	3.44	25.23
		<i>Cocconeis placentula</i>	8.52	3.96	2.61	3.27	28.49
		<i>Gomphonema affine</i>	3.88	0	2.27	2.85	31.34
		<i>Nitzschia desertorum</i>	0	3.92	2.25	2.82	34.17
		<i>Nitzschia recta</i>	0	3.79	2.18	2.73	36.89

Appendix B5 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Surirella brebissoni</i>	0	3.65	2.1	2.63	39.53
		<i>Gyrosigma attenuatum</i>	3.26	0	1.89	2.37	41.89
		<i>Nitzschia frustulum</i>	0.67	3.92	1.88	2.36	44.25
		<i>Aulacoseira granulata</i>	3.07	0	1.88	2.35	46.6
		<i>Amphora coffeaeformis</i>	3.09	0	1.73	2.16	48.76
		<i>Hantzschia distinctepunctata</i>	3.07	0	1.71	2.14	50.91
		<i>Rhopalodia gibba</i>	2.85	0	1.58	1.98	52.89
		<i>Navicula erifuga</i>	2.56	0	1.56	1.96	54.85
		<i>Epithemia sorex</i>	2.71	0	1.5	1.88	56.73
		<i>Surirella angusta</i>	0	2.58	1.48	1.86	58.59
		<i>Nitzschia palea</i>	3.08	5.6	1.45	1.81	60.41
		<i>Diploneis elliptica</i>	0	2.45	1.41	1.77	62.17
		<i>Navicula recens</i>	2.67	3.87	1.37	1.71	63.89
		<i>Navicula tripunctata</i>	0.88	3.06	1.26	1.58	65.47
		<i>Craticula buderi</i>	2.79	4.83	1.22	1.53	67.01
		<i>Cymbella tumida</i>	2.17	0	1.21	1.52	68.52
		<i>Gomphonema agustatum</i>	0.54	2.58	1.18	1.48	70
		<i>Tryblionella coarctata</i>	0	2	1.15	1.44	71.44
		<i>Tryblionella littoralis</i>	0	2	1.15	1.44	72.89
		<i>Sellaphora pupula</i>	0	1.83	1.05	1.32	74.2
		<i>Tryblionella apiculata</i>	1.41	3.11	1	1.26	75.46
		<i>Eolimna subminuscula</i>	0	1.73	1	1.25	76.71
		<i>Fragilaria biceps</i>	1.72	0	0.96	1.2	77.91
		<i>Cocconeis pediculus</i>	1.67	0	0.93	1.17	79.08
		<i>Rhopalodia gibberula</i>	1.49	0	0.91	1.14	80.22
		<i>Surirella tenera</i>	0	1.53	0.88	1.1	81.32
		<i>Nitzschia dissipata</i>	2.33	2.65	0.82	1.02	82.35
		<i>Mastogloia smithii</i>	1.37	0	0.81	1.02	83.37
		<i>Encyonema silesiacum</i>	1.29	0	0.79	0.99	84.36
		<i>Navicula veneta</i>	1.29	0	0.79	0.99	85.34
		<i>Nitzschia</i> sp.	1.29	0	0.79	0.99	86.33
		<i>Achnanthyidium exiguum</i>	1.33	0	0.74	0.93	87.26
		<i>Nitzschia amphibia</i>	0	1.29	0.74	0.93	88.19
		<i>Seminavis strigosa</i>	1.2	0	0.73	0.92	89.11
		<i>Fragilaria ulna</i> var. <i>acus</i>	1.05	0	0.64	0.81	89.92
		<i>Tryblionella gracilis</i>	1	0	0.56	0.7	90.62
			High	None			
High & none	64.02	<i>Pleurosigma salinarum</i>	1.77	7.96	3.41	5.33	5.33
		<i>Navicula microcari</i>	0	5	2.71	4.23	9.56
		<i>Craticula halophila</i>	2.69	7.53	2.62	4.1	13.65
		<i>Cocconeis placentula</i>	7.93	3.96	2.19	3.42	17.07
		<i>Nitzschia desertorum</i>	0	3.92	2.12	3.31	20.39
		<i>Tryblionella levidensis</i>	1.63	4.8	1.7	2.66	23.05
		<i>Nitzschia frustulum</i>	1.67	3.92	1.64	2.57	25.61
		<i>Craticula buderi</i>	2.13	4.83	1.5	2.34	27.95
		<i>Surirella brebissoni</i>	0.98	3.65	1.48	2.31	30.26
		<i>Navicula radiosa</i>	3.43	5.54	1.45	2.26	32.52
		<i>Surirella angusta</i>	0	2.58	1.4	2.18	34.71
		<i>Nitzschia dissipata</i>	1.7	2.65	1.4	2.18	36.89
		<i>Diploneis elliptica</i>	0	2.45	1.33	2.07	38.96
		<i>Epithemia sorex</i>	2.3	0	1.26	1.97	40.93
		<i>Navicula recens</i>	2.22	3.87	1.17	1.82	42.75
		<i>Nitzschia sigma</i>	2.06	0	1.16	1.82	44.57
		<i>Navicula tripunctata</i>	1.11	3.06	1.14	1.79	46.35
		<i>Gomphonema agustatum</i>	1.22	2.58	1.14	1.79	48.14
		<i>Hantzschia distinctepunctata</i>	2.21	0	1.14	1.78	49.92
		<i>Navicula veneta</i>	2.04	0	1.12	1.75	51.67
		<i>Gyrosigma attenuatum</i>	1.95	0	1.1	1.72	53.39
		<i>Navicula</i> sp.	1.91	0	1.09	1.71	55.09
		<i>Tryblionella coarctata</i>	0	2	1.08	1.69	56.79
		<i>Fragilaria biceps</i>	2.05	0	1.07	1.66	58.45

Appendix B5 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Sellaphora pupula</i>	0	1.83	0.99	1.54	59.99
		<i>Nitzschia</i> sp.	1.67	0	0.95	1.48	61.48
		<i>Eolimna subminuscula</i>	1.15	1.73	0.94	1.46	62.94
		<i>Cyclotella meneghiniana</i>	1.45	0.58	0.92	1.44	64.38
		<i>Gomphonema</i> sp.	1.6	0	0.91	1.42	65.81
		<i>Nitzschia filiformis</i>	1.56	0	0.89	1.39	67.2
		<i>Amphora coffeaeformis</i>	1.6	0	0.84	1.31	68.51
		<i>Navicula erifuga</i>	1.45	0	0.83	1.29	69.8
		<i>Surirella tenera</i>	0	1.53	0.83	1.29	71.09
		<i>Discostella stelligera</i>	1.63	0	0.83	1.29	72.38
		<i>Nitzschia recta</i>	2.75	3.79	0.82	1.28	73.67
		<i>Tryblionella littoralis</i>	0.79	2	0.81	1.26	74.93
		<i>Hippodonta</i> sp.	1.29	0	0.74	1.15	76.08
		<i>Planothidium engelbrechtii</i>	1.32	0	0.72	1.13	77.21
		<i>Fragilaria ulna</i> var. <i>acus</i>	1.43	0	0.72	1.13	78.34
		<i>Diploneis subovalis</i>	1.4	0	0.71	1.11	79.45
		<i>Nitzschia amphibia</i>	0	1.29	0.7	1.09	80.54
		<i>Tryblionella apiculata</i>	2.7	3.11	0.68	1.06	81.6
		<i>Nitzschia pumila</i>	1.25	0	0.63	0.99	82.59
		<i>Cocconeis pediculus</i>	1.14	0	0.62	0.97	83.56
		<i>Nitzschia linearis</i>	1.22	0	0.62	0.96	84.53
		<i>Rhopalodia gibba</i>	1.12	0	0.61	0.96	85.49
		<i>Navicula symmetrica</i>	1.09	0	0.6	0.93	86.42
		<i>Nitzschia reversa</i>	1.07	0	0.54	0.85	87.26
		<i>Gomphonema parvulum</i>	1.02	0	0.54	0.84	88.1
		<i>Cymbella aspera</i>	1.04	0	0.52	0.82	88.92
		<i>Rhopalodia operculata</i>	0.92	0	0.51	0.79	89.71
		<i>Nitzschia closterium</i>	0.98	0	0.5	0.78	90.49
		Low		None			
		<i>Craticula halophila</i>	1.69	7.53	3.08	5.23	5.23
		<i>Tryblionella levidensis</i>	0	4.8	2.51	4.26	9.49
		<i>Pleurosigma salinarum</i>	4.49	7.96	2.21	3.76	13.25
		<i>Navicula microcari</i>	1.29	5	1.97	3.34	16.59
		<i>Navicula tripunctata</i>	2.61	3.06	1.91	3.25	19.84
		<i>Nitzschia frustulum</i>	0.82	3.92	1.63	2.77	22.61
		<i>Nitzschia palea</i>	2.61	5.6	1.54	2.61	25.22
		<i>Nitzschia desertorum</i>	1.04	3.92	1.53	2.59	27.81
		<i>Hantzschia distinctepunctata</i>	2.96	0	1.52	2.59	30.4
		<i>Navicula radiosa</i>	2.75	5.54	1.5	2.55	32.94
		<i>Diploneis elliptica</i>	1.95	2.45	1.44	2.45	35.4
		<i>Navicula cryptotenella</i>	2.77	0	1.41	2.4	37.8
		<i>Surirella brebissoni</i>	1.11	3.65	1.35	2.3	40.09
		<i>Surirella angusta</i>	0	2.58	1.35	2.3	42.39
		<i>Fragilaria ulna</i> var. <i>acus</i>	2.53	0	1.35	2.29	44.68
		<i>Cymbella turgidula</i>	2.57	0	1.33	2.26	46.94
		<i>Cyclotella meneghiniana</i>	2.34	0.58	1.14	1.93	48.87
		<i>Navicula recens</i>	2.32	3.87	1.12	1.9	50.77
		<i>Cymbella tumida</i>	2.13	0	1.09	1.86	52.62
		<i>Cymbella kolbei</i>	2.04	0	1.05	1.79	54.41
		<i>Gomphonema agustatum</i>	1.11	2.58	1.03	1.76	56.16
		<i>Achnanthydium eutrophilum</i>	1.91	0	1.03	1.75	57.92
		<i>Craticula buderi</i>	6.74	4.83	1.02	1.73	59.64
		<i>Fragilaria biceps</i>	1.91	0	0.98	1.66	61.3
		<i>Gyrosigma attenuatum</i>	1.9	0	0.97	1.65	62.95
		<i>Nitzschia dissipata</i>	2.04	2.65	0.84	1.43	64.39
		<i>Navicula antonii</i>	1.53	0	0.84	1.42	65.81
		<i>Gomphonema parvulum</i>	1.59	0	0.81	1.38	67.19
		<i>Tryblionella littoralis</i>	0.9	2	0.81	1.38	68.56
		<i>Cymbella neocistula</i>	1.54	0	0.79	1.35	69.91
		<i>Amphora coffeaeformis</i>	1.48	0	0.76	1.29	71.2
		<i>Tryblionella coarctata</i>	0.64	2	0.72	1.23	72.43
Low & none	58.89						

Appendix B5 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Nitzschia umbonata</i>	1.38	0	0.72	1.22	73.65
		<i>Craticula cuspidata</i>	1.31	0	0.7	1.19	74.83
		<i>Cocconeis placentula</i>	3.77	3.96	0.69	1.17	76
		<i>Nitzschia amphibia</i>	0	1.29	0.68	1.15	77.15
		<i>Cymbella aspera</i>	1.29	0	0.67	1.13	78.28
		<i>Encyonema minutum</i>	1.3	0	0.66	1.13	79.41
		<i>Tryblionella apiculata</i>	2.25	3.11	0.65	1.11	80.52
		<i>Surirella tenera</i>	0.72	1.53	0.65	1.1	81.62
		<i>Eolimna subminuscula</i>	0.47	1.73	0.65	1.1	82.72
		<i>Seminavis strigosa</i>	1.2	0	0.61	1.03	83.75
		<i>Fallacia monoculata</i>	1.15	0	0.58	0.99	84.75
		<i>Eolimna minima</i>	1.11	0	0.57	0.97	85.71
		<i>Fragilaria ulna</i>	1.09	0	0.55	0.94	86.65
		<i>Luticola mutica</i>	0.96	0	0.49	0.84	87.49
		<i>Caloneis molaris</i>	0.88	0	0.46	0.77	88.26
		<i>Craticula ambigua</i>	0.82	0	0.45	0.76	89.02
		<i>Surirella ovalis</i>	0.88	0	0.45	0.76	89.78
		<i>Gomphonema lagenula</i>	0.79	0	0.44	0.74	90.52

Appendix B6: Contribution of various diatom species within group similarities determined through a Similarity percentage analysis (SIMPER). Groups are based on rivers sampled (Phongolo River 2017, Olifants River 2020 and 2021 or Limpopo River 2021) and were determined using Bray Curtis similarity matrix.

Rivers	Overall similarity (%)	Species	Average abundance	Average similarity	Contribution (%)	Cumulative contribution (%)
Phongolo	39	<i>Cocconeis placentula</i>	8.82	11	28.19	28.19
		<i>Nitzschia</i> sp.	5.28	6.35	16.28	44.48
		<i>Navicula</i> sp.	4.5	3.73	9.57	54.05
		<i>Navicula erifuga</i>	4.31	3.21	8.22	62.27
		<i>Gomphonema</i> sp.	3.31	2.84	7.29	69.56
		<i>Fragilaria ulna</i> var. <i>acus</i>	3.45	2.79	7.15	76.71
		<i>Nitzschia palea</i>	3.02	2.57	6.59	83.3
		<i>Aulacoseira granulata</i>	4.65	2.2	5.65	88.95
		<i>Tryblionella apiculata</i>	2.24	0.99	2.54	91.49
Olifants	38.11	<i>Cocconeis placentula</i>	7.77	6.33	16.62	16.62
		<i>Craticula buderi</i>	4.09	3.67	9.63	26.25
		<i>Hantzschia distinctepunctata</i>	4.5	3.54	9.29	35.54
		<i>Amphora coffeaeformis</i>	3.36	2.7	7.09	42.63
		<i>Nitzschia palea</i>	4.1	2.67	7.01	49.64
		<i>Navicula recens</i>	3.37	2.59	6.79	56.44
		<i>Fragilaria biceps</i>	2.92	2.51	6.58	63.02
		<i>Cocconeis pediculus</i>	2.01	1.29	3.4	66.42
		<i>Gyrosigma attenuatum</i>	2.22	1.23	3.23	69.65
		<i>Tryblionella apiculata</i>	1.59	1.03	2.7	72.35
		<i>Navicula radiosa</i>	2.62	0.98	2.56	74.91
		<i>Cymbella tumida</i>	2.18	0.97	2.56	77.47
		<i>Gomphonema minutum</i>	2.37	0.88	2.32	79.79
		<i>Epithemia sorex</i>	3.01	0.77	2.02	81.81
		<i>Craticula halophila</i>	1.72	0.68	1.77	83.59
		<i>Gomphonema agustatum</i>	1.72	0.66	1.73	85.32
		<i>Cymbella aspera</i>	1.72	0.64	1.68	87
		<i>Nitzschia frustulum</i>	1.66	0.47	1.23	88.23
		<i>Gomphonema parvulum</i>	1.01	0.45	1.18	89.4
		<i>Amphora veneta</i>	1.02	0.4	1.05	90.46

Appendix B6 continued.

Rivers	Overall similarity (%)	Species	Average abundance	Average similarity	Contribution (%)	Cumulative contribution (%)
Limpopo	40.38	<i>Pleurosigma salinarum</i>	7.14	5.81	14.38	14.38
		<i>Craticula buderii</i>	6.45	5.52	13.66	28.04
		<i>Nitzschia recta</i>	4.34	4.2	10.41	38.45
		<i>Tryblionella apiculata</i>	3.29	3.2	7.94	46.39
		<i>Cocconeis placentula</i>	3.59	2.56	6.33	52.72
		<i>Navicula radiosa</i>	3.65	1.83	4.52	57.24
		<i>Sellaphora pupula</i>	1.92	1.77	4.38	61.62
		<i>Nitzschia palea</i>	3.42	1.7	4.22	65.83
		<i>Navicula recens</i>	2.89	1.42	3.51	69.34
		<i>Cyclotella meneghiniana</i>	2.53	1.39	3.43	72.77
		<i>Navicula microcari</i>	2.96	1.31	3.24	76.01
		<i>Surirella brebissoni</i>	2.32	1.12	2.77	78.78
		<i>Navicula tripunctata</i>	3.63	1.12	2.77	81.55
		<i>Nitzschia desertorum</i>	2.34	1.05	2.6	84.14
		<i>Nitzschia dissipata</i>	2.26	0.89	2.21	86.35
		<i>Diploneis elliptica</i>	2.77	0.83	2.05	88.4
		<i>Tryblionella littoralis</i>	1.57	0.73	1.81	90.21

Appendix B7: Contribution of various diatom species within pairwise group dissimilarities determined through a Similarity percentage analysis (SIMPER). Groups are based on rivers sampled (Phongolo River 2017, Olifants River 2020 and 2021 or Limpopo River 2021) and were determined using Bray Curtis similarity matrix.

Rivers	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
			Phongolo	Olifants			
Phongolo & Olifants	80.29	<i>Nitzschia</i> sp.	5.28	0	3.21	3.99	3.99
		<i>Aulacoseira granulata</i>	4.65	0	2.83	3.53	7.52
		<i>Navicula</i> sp.	4.5	0	2.73	3.4	10.92
		<i>Hantzschia distinctepunctata</i>	0	4.5	2.69	3.35	14.27
		<i>Navicula erifuga</i>	4.31	0	2.59	3.23	17.5
		<i>Craticula buderii</i>	0	4.09	2.46	3.07	20.56
		<i>Navicula recens</i>	0	3.37	2.06	2.57	23.13
		<i>Gomphonema</i> sp.	3.31	0	1.99	2.48	25.61
		<i>Epithemia sores</i>	0	3.01	1.87	2.33	27.94
		<i>Amphora coffeaeformis</i>	1.15	3.36	1.77	2.21	30.15
		<i>Fragilaria ulna</i> var. <i>acus</i>	3.45	1.54	1.75	2.18	32.33
		<i>Fragilaria biceps</i>	0	2.92	1.74	2.17	34.5
		<i>Cocconeis placentula</i>	8.82	7.77	1.62	2.02	36.52
		<i>Nitzschia palea</i>	3.02	4.1	1.57	1.95	38.47
		<i>Navicula radiosa</i>	1.17	2.62	1.56	1.95	40.42
		<i>Gomphonema minutum</i>	1.17	2.37	1.55	1.93	42.35
		<i>Rhopalodia gibba</i>	0	2.38	1.49	1.85	44.2
		<i>Tryblionella apiculata</i>	2.24	1.59	1.35	1.68	45.88
		<i>Cymbella tumida</i>	0	2.18	1.33	1.66	47.53
		<i>Cyclotella meneghiniana</i>	2.21	0	1.3	1.62	49.15
		<i>Gomphonema affine</i>	1.56	1.08	1.28	1.59	50.75
		<i>Gyrosigma attenuatum</i>	2.06	2.22	1.26	1.57	52.32
		<i>Nitzschia dissipata</i>	2.02	1.2	1.26	1.56	53.89
		<i>Cocconeis pediculus</i>	0	2.01	1.24	1.55	55.43
		<i>Navicula veneta</i>	2	0.4	1.17	1.45	56.89
		<i>Craticula halophila</i>	1.09	1.72	1.15	1.43	58.31
		<i>Nitzschia recta</i>	1.12	1.67	1.14	1.42	59.74
		<i>Cymbella aspera</i>	0	1.72	1	1.25	60.99
		<i>Gomphonema agustatum</i>	0	1.72	1	1.25	62.24
		<i>Nitzschia frustulum</i>	0	1.66	0.97	1.2	63.44
		<i>Cymbella turgidula</i>	0	1.54	0.89	1.11	64.55

Appendix B7 continued.

Rivers	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Navicula rostellata</i>	1.35	0	0.85	1.06	65.62
		<i>Nitzschia siliqua</i>	1.32	0	0.84	1.05	66.66
		<i>Gomphonema parvulum</i>	0.87	1.01	0.82	1.03	67.69
		<i>Nitzschia sigma</i>	1.09	0.57	0.8	1	68.68
		<i>Fragilaria tenera</i>	1.25	0	0.79	0.99	69.67
		<i>Navicula tripunctata</i>	0	1.19	0.74	0.93	70.6
		<i>Navicula symmetrica</i>	0	1.19	0.74	0.93	71.52
		<i>Cymbella kolbei</i>	0	1.22	0.71	0.88	72.4
		<i>Tryblionella levidensis</i>	1.22	0	0.69	0.86	73.26
		<i>Achnanthydium</i> sp.	1.06	0	0.67	0.84	74.1
		<i>Rhopalodia gibberula</i>	1.12	0	0.67	0.84	74.93
		<i>Nitzschia filiformis</i>	1.17	0	0.66	0.82	75.75
		<i>Encyonema silesiacum</i>	0.97	0.16	0.63	0.78	76.54
		<i>Amphora veneta</i>	0	1.02	0.63	0.78	77.32
		<i>Pleurosigma salinarum</i>	0	1.06	0.6	0.75	78.06
		<i>Discostella stelligera</i>	0	0.98	0.55	0.69	78.75
		<i>Nitzschia umbonata</i>	0	0.92	0.55	0.68	79.44
		<i>Hippodonta</i> sp.	0.97	0	0.54	0.68	80.11
		<i>Seminavis strigosa</i>	0.9	0	0.54	0.67	80.79
		<i>Gyrosigma</i> sp.	0.87	0	0.54	0.67	81.45
		<i>Cymbella neocistula</i>	0	0.92	0.53	0.66	82.12
		<i>Rhopalodia operculata</i>	0	0.88	0.53	0.66	82.78
		<i>Amphora pediculus</i>	0	0.82	0.51	0.64	83.41
		<i>Mastogloia smithii</i>	0.71	0.26	0.51	0.63	84.04
		<i>Achnanthydium exiguum</i>	0	0.8	0.5	0.63	84.67
		<i>Planothidium engelbrechtii</i>	0	0.79	0.49	0.61	85.28
		<i>Eolimna subminuscula</i>	0.87	0	0.49	0.61	85.89
		<i>Gomphonema pseudoaugur</i>	0	0.77	0.48	0.59	86.48
		<i>Diploneis subovalis</i>	0	0.84	0.47	0.59	87.07
		<i>Nitzschia pumila</i>	0	0.75	0.42	0.53	87.6
		<i>Nitzschia obtusa</i>	0	0.68	0.41	0.52	88.11
		<i>Achnanthydium eutrophilum</i>	0	0.69	0.41	0.51	88.63
		<i>Nitzschia linearis</i>	0	0.73	0.41	0.51	89.14
		<i>Capartogramma crucicula</i>	0.66	0	0.41	0.51	89.65
		<i>Eolimna minima</i>	0	0.66	0.38	0.48	90.12
Phongolo & Limpopo	82.61		Phongolo	Limpopo			
		<i>Pleurosigma salinarum</i>	0	7.14	4.33	5.24	5.24
		<i>Craticula buderi</i>	0	6.45	3.87	4.69	9.92
		<i>Cocconeis placentula</i>	8.82	3.59	3.2	3.87	13.79
		<i>Nitzschia</i> sp.	5.28	0	3.18	3.85	17.64
		<i>Aulacoseira granulata</i>	4.65	0	2.81	3.4	21.05
		<i>Navicula</i> sp.	4.5	0	2.71	3.28	24.32
		<i>Navicula erifuga</i>	4.31	0	2.57	3.11	27.43
		<i>Navicula tripunctata</i>	0	3.63	2.24	2.71	30.14
		<i>Nitzschia recta</i>	1.12	4.34	2.04	2.47	32.61
		<i>Gomphonema</i> sp.	3.31	0	1.98	2.39	35
		<i>Craticula halophila</i>	1.09	3.18	1.93	2.34	37.34
		<i>Navicula radiosa</i>	1.17	3.65	1.93	2.33	39.68
		<i>Navicula recens</i>	0	2.89	1.78	2.16	41.83
		<i>Fragilaria ulna</i> var. <i>acus</i>	3.45	1.39	1.77	2.14	43.97
		<i>Navicula microcari</i>	0	2.96	1.75	2.12	46.09
		<i>Diploneis elliptica</i>	0	2.77	1.6	1.94	48.03
		<i>Navicula cryptotenella</i>	0	2.77	1.58	1.91	49.94
		<i>Nitzschia palea</i>	3.02	3.42	1.45	1.75	51.69
		<i>Nitzschia desertorum</i>	0	2.34	1.39	1.68	53.37
		<i>Surirella brebissoni</i>	0	2.32	1.37	1.66	55.03
		<i>Tryblionella apiculata</i>	2.24	3.29	1.35	1.63	56.66
		<i>Cyclotella meneghiniana</i>	2.21	2.53	1.33	1.61	58.27
		<i>Nitzschia dissipata</i>	2.02	2.26	1.31	1.59	59.86
		<i>Tryblionella levidensis</i>	1.22	1.6	1.21	1.46	61.32
		<i>Gyrosigma attenuatum</i>	2.06	0.67	1.2	1.46	62.77

Appendix B7 continued.

Rivers	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Navicula veneta</i>	2	0	1.15	1.39	64.17
		<i>Sellaphora pupula</i>	0	1.92	1.14	1.39	65.55
		<i>Gomphonema affine</i>	1.56	0.54	1.1	1.33	66.88
		<i>Nitzschia frustulum</i>	0	1.69	1.02	1.23	68.12
		<i>Tryblionella littoralis</i>	0	1.57	0.97	1.17	69.29
		<i>Navicula antonii</i>	0	1.53	0.94	1.14	70.43
		<i>Seminavis strigosa</i>	0.9	1.2	0.88	1.06	71.49
		<i>Achnantheidium eutrophilum</i>	0	1.4	0.87	1.05	72.54
		<i>Amphora coffeaeformis</i>	1.15	0.58	0.86	1.04	73.59
		<i>Navicula rostellata</i>	1.35	0	0.85	1.03	74.61
		<i>Nitzschia siliqua</i>	1.32	0	0.83	1.01	75.62
		<i>Eolimna subminuscula</i>	0.87	1.05	0.83	1	76.62
		<i>Nitzschia umbonata</i>	0	1.38	0.8	0.97	77.59
		<i>Nitzschia filiformis</i>	1.17	0.43	0.8	0.96	78.56
		<i>Fragilaria tenera</i>	1.25	0	0.79	0.95	79.51
		<i>Gomphonema parvulum</i>	0.87	0.92	0.79	0.95	80.46
		<i>Craticula cuspidata</i>	0	1.31	0.78	0.95	81.41
		<i>Tryblionella coarctata</i>	0	1.3	0.77	0.93	82.34
		<i>Surirella tenera</i>	0	1.23	0.72	0.87	83.21
		<i>Gomphonema minutum</i>	1.17	0	0.7	0.85	84.06
		<i>Achnantheidium sp.</i>	1.06	0	0.67	0.81	84.87
		<i>Rhopalodia gibberula</i>	1.12	0	0.67	0.81	85.67
		<i>Fallacia monoculata</i>	0	1.15	0.65	0.79	86.46
		<i>Fragilaria ulna</i>	0	1.09	0.61	0.74	87.21
		<i>Nitzschia sigma</i>	1.09	0	0.61	0.74	87.94
		<i>Encyonema silesiacum</i>	0.97	0	0.58	0.7	88.64
		<i>Hippodonta sp.</i>	0.97	0	0.54	0.65	89.3
		<i>Gyrosigma sp.</i>	0.87	0	0.53	0.64	89.94
		<i>Gomphonema angustatum</i>	0	0.86	0.53	0.64	90.58
Olifants & Limpopo	71.17		Olifants	Limpopo			
		<i>Pleurosigma salinarum</i>	1.06	7.14	3.33	4.68	4.68
		<i>Cocconeis placentula</i>	7.77	3.59	2.31	3.25	7.92
		<i>Hantzschia distinctepunctata</i>	4.5	0.75	2.02	2.84	10.76
		<i>Navicula tripunctata</i>	1.19	3.63	1.74	2.44	13.21
		<i>Epithemia sorex</i>	3.01	0	1.62	2.28	15.49
		<i>Craticula halophila</i>	1.72	3.18	1.58	2.22	17.7
		<i>Navicula radiosa</i>	2.62	3.65	1.57	2.21	19.91
		<i>Navicula microcari</i>	0	2.96	1.54	2.16	22.07
		<i>Nitzschia recta</i>	1.67	4.34	1.5	2.1	24.17
		<i>Amphora coffeaeformis</i>	3.36	0.58	1.49	2.09	26.27
		<i>Diploneis elliptica</i>	0	2.77	1.41	1.98	28.25
		<i>Navicula cryptotenella</i>	0.35	2.77	1.39	1.95	30.2
		<i>Nitzschia palea</i>	4.1	3.42	1.37	1.92	32.12
		<i>Cyclotella meneghiniana</i>	0	2.53	1.33	1.86	33.99
		<i>Craticula buderii</i>	4.09	6.45	1.31	1.84	35.83
		<i>Rhopalodia gibba</i>	2.38	0	1.29	1.81	37.64
		<i>Gomphonema minutum</i>	2.37	0	1.29	1.81	39.45
		<i>Nitzschia desertorum</i>	0	2.34	1.22	1.71	41.16
		<i>Fragilaria biceps</i>	2.92	0.82	1.15	1.61	42.77
		<i>Navicula recens</i>	3.37	2.89	1.14	1.6	44.37
		<i>Surirella brebissoni</i>	0.59	2.32	1.11	1.56	45.93
		<i>Cymbella tumida</i>	2.18	0.67	1.08	1.52	47.46
		<i>Cocconeis pediculus</i>	2.01	0	1.08	1.52	48.97
		<i>Nitzschia dissipata</i>	1.2	2.26	1.06	1.49	50.46
		<i>Gyrosigma attenuatum</i>	2.22	0.67	1.03	1.44	51.9
		<i>Nitzschia frustulum</i>	1.66	1.69	1.01	1.42	53.32
		<i>Sellaphora pupula</i>	0	1.92	1	1.41	54.73
		<i>Fragilaria ulna</i> var. <i>acus</i>	1.54	1.39	1	1.41	56.14
		<i>Tryblionella apiculata</i>	1.59	3.29	0.89	1.25	57.39
		<i>Cymbella aspera</i>	1.72	0	0.88	1.23	58.62
		<i>Achnantheidium eutrophilum</i>	0.69	1.4	0.87	1.22	59.84

Appendix B7 continued.

Rivers	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Gomphonema agustatum</i>	1.72	0.86	0.86	1.21	61.06
		<i>Tryblionella levidensis</i>	0	1.6	0.86	1.21	62.26
		<i>Navicula antonii</i>	0	1.53	0.83	1.16	63.42
		<i>Tryblionella littoralis</i>	0.48	1.57	0.79	1.1	64.53
		<i>Cymbella turgidula</i>	1.54	0	0.78	1.1	65.62
		<i>Nitzschia umbonata</i>	0.92	1.38	0.72	1.01	66.63
		<i>Craticula cuspidata</i>	0	1.31	0.69	0.97	67.6
		<i>Tryblionella coarctata</i>	0	1.3	0.68	0.95	68.55
		<i>Gomphonema parvulum</i>	1.01	0.92	0.65	0.91	69.46
		<i>Navicula symmetrica</i>	1.19	0	0.64	0.9	70.37
		<i>Gomphonema affine</i>	1.08	0.54	0.64	0.9	71.26
		<i>Surirella tenera</i>	0	1.23	0.63	0.89	72.15
		<i>Fragilaria ulna</i>	0.42	1.09	0.63	0.88	73.03
		<i>Cymbella kolbei</i>	1.22	0	0.62	0.87	73.9
		<i>Seminavis strigosa</i>	0	1.2	0.6	0.84	74.74
		<i>Fallacia monoculata</i>	0	1.15	0.58	0.81	75.55
		<i>Eolimna subminuscula</i>	0	1.05	0.56	0.79	76.35
		<i>Amphora veneta</i>	1.02	0	0.54	0.76	77.11
		<i>Discostella stelligera</i>	0.98	0	0.49	0.68	77.79
		<i>Gomphonema lagenula</i>	0.28	0.79	0.48	0.67	78.46
		<i>Cymbella neocistula</i>	0.92	0	0.47	0.66	79.12
		<i>Surirella angusta</i>	0	0.86	0.46	0.65	79.77
		<i>Rhopalodia operculata</i>	0.88	0	0.46	0.65	80.42
		<i>Amphora pediculus</i>	0.82	0	0.44	0.62	81.04
		<i>Craticula ambigua</i>	0	0.82	0.44	0.62	81.66
		<i>Surirella ovalis</i>	0	0.88	0.44	0.62	82.28
		<i>Nitzschia reversa</i>	0.64	0.38	0.44	0.62	82.89
		<i>Achnanthisidium exiguum</i>	0.8	0	0.44	0.61	83.5
		<i>Tryblionella hungarica</i>	0	0.79	0.43	0.6	84.11
		<i>Planothidium engelbrechtii</i>	0.79	0	0.42	0.6	84.7
		<i>Diploneis subovalis</i>	0.84	0	0.42	0.59	85.29
		<i>Gomphonema pseudoaugur</i>	0.77	0	0.41	0.58	85.87
		<i>Nitzschia closterium</i>	0.59	0.33	0.4	0.57	86.44
		<i>Nitzschia obtusa</i>	0.68	0.38	0.4	0.56	87
		<i>Encyonema minutum</i>	0.43	0.58	0.4	0.56	87.56
		<i>Nitzschia pumila</i>	0.75	0	0.37	0.52	88.08
		<i>Nitzschia linearis</i>	0.73	0	0.36	0.51	88.58
		<i>Eolimna minima</i>	0.66	0	0.34	0.47	89.06
		<i>Tryblionella gracilis</i>	0.6	0	0.33	0.46	89.51
		<i>Amphora fontinalis</i>	0.57	0	0.31	0.43	89.95
		<i>Nitzschia sigma</i>	0.57	0	0.3	0.43	90.38

Appendix B8: Contribution of various diatom functional groups within group similarities determined through a Similarity percentage analysis (SIMPER). Groups are based on *T. granifera* density sampled at sites on the Phongolo River 2017, Olifants River 2020 and 2021 or Limpopo River 2021 and were determined using Bray Curtis similarity matrix.

<i>T. granifera</i> density	Overall similarity (%)	Classification guilds	Average abundance	Average similarity	Contribution (%)	Cumulative contribution (%)
Very high	81.6	Mobile	9.45	8.47	10.38	10.38
		Biraphideae	8.35	7.6	9.31	19.69
		Intermediate colonists	7.7	6.6	8.09	27.78
		Motile	7.02	6.11	7.49	35.27
		Size 5	7.22	6.09	7.46	42.74
		Low profile	5.48	4.74	5.81	48.55
		Pioneer colonists	5.68	4.44	5.44	53.99
		LS5	5.1	4.29	5.26	59.25
		Size 4	4.5	4	4.9	64.14

Appendix B8 continued.

<i>T. granifera</i> density	Overall similarity (%)	Classification guilds	Average abundance	Average similarity	Contribution (%)	Cumulative contribution (%)
High	81.23	Monoraphideae	4.52	3.94	4.83	68.98
		High profile	4.4	3.63	4.44	73.42
		MS4	3.73	3.16	3.87	77.3
		Size 3	4.18	2.72	3.33	80.62
		Late colonists	2.48	2.04	2.51	83.13
		Size 2	2.36	1.96	2.4	85.53
		HS3	2.65	1.96	2.4	87.93
		MS2	2.2	1.88	2.3	90.23
		Mobile	9.81	9.25	11.39	11.39
		Biraphideae	8.53	7.71	9.5	20.88
		Intermediate colonists	8.16	7.53	9.27	30.15
		Motile	8.06	7.1	8.74	38.89
		Size 5	6.32	5.04	6.2	45.09
		Size 3	4.77	4.18	5.15	50.24
		Size 4	5.15	4.09	5.04	55.28
		Low profile	4.73	3.59	4.42	59.7
		MS4	4.68	3.56	4.39	64.09
		MS3	4	3.32	4.09	68.18
		Pioneer colonists	4.42	3.32	4.09	72.26
		MS5	4.04	3.3	4.07	76.33
		LS5	4.21	3.14	3.87	80.19
		Late colonists	3.46	2.87	3.53	83.72
		Monoraphideae	4.15	2.71	3.33	87.06
		High profile	3.08	2.39	2.94	90
		Size 2	2.61	1.85	2.28	92.27
Medium	80.09	Mobile	8.97	8.1	10.11	10.11
		Intermediate colonists	8.29	7.6	9.49	19.6
		Motile	7.12	6.54	8.16	27.76
		Biraphideae	6.93	6.39	7.98	35.74
		Low profile	6.36	6.08	7.6	43.34
		LS5	6.15	5.66	7.07	50.41
		Size 5	6.51	5.3	6.62	57.03
		Size 3	5.63	5.26	6.57	63.6
		Monoraphideae	5.26	4.63	5.78	69.38
		MS3	4.83	4.22	5.27	74.65
		Pioneer colonists	5.21	4.01	5.01	79.66
		Size 4	4.33	3.57	4.46	84.12
		MS4	4.47	3.5	4.37	88.49
		Araphideae	3.19	2.82	3.52	92.01
Low	84.92	Mobile	9.72	9.05	10.66	10.66
		Biraphideae	9.34	8.71	10.26	20.92
		Motile	8.09	6.74	7.93	28.85
		Intermediate colonists	7.19	6.2	7.29	36.14
		Size 5	6.26	5.12	6.03	42.17
		Pioneer colonists	5.39	4.68	5.51	47.69
		Size 4	4.89	4.07	4.8	52.48
		MS4	5	4.01	4.72	57.2
		Size 3	4.3	3.69	4.34	61.55
		Late colonists	4.25	3.52	4.15	65.7
		Size 2	3.73	3.17	3.73	69.43
		High profile	4.65	3.07	3.61	73.04
		MS2	3.88	3.06	3.61	76.65
		MS3	3.74	2.95	3.47	80.12
		Low profile	2.93	2.5	2.94	83.07
		MS5	2.97	2.19	2.58	85.65
		Monoraphideae	2.41	2.16	2.54	88.19
		Colonial	2.33	2.02	2.38	90.57

Appendix B9: Contribution of various diatom functional groups within pairwise group dissimilarities determined through a Similarity percentage analysis (SIMPER). Groups are based on *T. granifera* density sampled at sites on the Phongolo River 2017, Olifants River 2020 and 2021 or Limpopo River 2021 and were determined using Bray Curtis similarity matrix.

<i>T. granifera</i> density	Overall dissimilarity (%)	Classification guilds	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
			Medium	Very high			
Medium & very high	19.28	MS5	1.81	3.08	1.28	6.66	6.66
		Centric	2.6	1.54	1.25	6.47	13.12
		HS4	0	2.4	1.14	5.94	19.06
		HS5	0	2.31	1.12	5.81	24.87
		MS3	4.83	2.93	1.1	5.69	30.56
		Colonial	4.09	2.73	1.08	5.58	36.14
		MS2	0	2.2	1.05	5.47	41.61
		High profile	2.81	4.4	0.8	4.14	45.75
		Araphideae	3.19	1.54	0.79	4.12	49.87
		Size 3	5.63	4.18	0.77	3.98	53.85
		Late colonists	1.15	2.48	0.7	3.62	57.47
		Size 5	6.51	7.22	0.69	3.58	61.05
		Biraphideae	6.93	8.35	0.68	3.53	64.58
		Pioneer colonists	5.21	5.68	0.65	3.36	67.94
		Size 2	1.25	2.36	0.64	3.32	71.26
		LS5	6.15	5.1	0.55	2.83	74.09
		LS1	1.13	0.67	0.54	2.82	76.91
		Size 1	1.06	0.69	0.51	2.64	79.55
		MS4	4.47	3.73	0.49	2.53	82.09
		Intermediate colonists	8.29	7.7	0.44	2.3	84.38
		HS3	2.81	2.65	0.42	2.2	86.58
		Low profile	6.36	5.48	0.42	2.19	88.77
		Mobile	8.97	9.45	0.41	2.11	90.89
Medium & high	20.48		Medium	High			
		Colonial	4.09	1.44	1.38	6.74	6.74
		Centric	2.6	1.53	1.26	6.15	12.88
		MS2	0	2.53	1.22	5.98	18.86
		MS5	1.81	4.04	1.17	5.7	24.56
		Late colonists	1.15	3.46	1.13	5.51	30.08
		HS5	0	2.11	1.01	4.93	35.01
		LS5	6.15	4.21	0.95	4.64	39.65
		Araphideae	3.19	1.3	0.94	4.58	44.23
		Monoraphideae	5.26	4.15	0.91	4.43	48.66
		Low profile	6.36	4.73	0.89	4.36	53.02
		Size 2	1.25	2.61	0.81	3.96	56.98
		Biraphideae	6.93	8.53	0.78	3.79	60.77
		Pioneer colonists	5.21	4.42	0.72	3.53	64.3
		Size 5	6.51	6.32	0.69	3.35	67.65
		MS4	4.47	4.68	0.61	3	70.65
		Size 4	4.33	5.15	0.61	2.96	73.61
		Motile	7.12	8.06	0.55	2.7	76.31
		LS1	1.13	0.3	0.55	2.68	78.99
		LS4	0	1.13	0.54	2.63	81.63
		MS3	4.83	4	0.52	2.53	84.16
		Size 1	1.06	0.32	0.51	2.51	86.67
		High profile	2.81	3.08	0.49	2.38	89.05
		HS3	2.81	2.34	0.48	2.35	91.4
Very high & high	17.67		Very high	High			
		MS5	3.08	4.04	1.12	6.33	6.33
		Centric	1.54	1.53	0.97	5.48	11.81
		MS3	2.93	4	0.95	5.36	17.17
		Colonial	2.73	1.44	0.94	5.3	22.46
		HS5	2.31	2.11	0.86	4.85	27.31
		HS4	2.4	0.59	0.86	4.84	32.15
		Pioneer colonists	5.68	4.42	0.82	4.65	36.81
		Monoraphideae	4.52	4.15	0.77	4.36	41.16
		Low profile	5.48	4.73	0.73	4.16	45.32

Appendix B9 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Classification guilds	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		Size 5	7.22	6.32	0.72	4.07	49.39
		High profile	4.4	3.08	0.72	4.05	53.44
		Size 3	4.18	4.77	0.69	3.91	57.35
		LS5	5.1	4.21	0.68	3.86	61.21
		MS4	3.73	4.68	0.61	3.43	64.64
		Motile	7.02	8.06	0.59	3.35	67.99
		Araphideae	1.54	1.3	0.56	3.18	71.17
		Size 4	4.5	5.15	0.55	3.12	74.29
		LS4	0	1.13	0.53	2.99	77.28
		Late colonists	2.48	3.46	0.47	2.66	79.94
		LS3	0.69	0.67	0.43	2.43	82.37
		Intermediate colonists	7.7	8.16	0.4	2.28	84.65
		HS3	2.65	2.34	0.4	2.26	86.91
		MS2	2.2	2.53	0.39	2.21	89.12
		Size 2	2.36	2.61	0.38	2.15	91.27
Medium & low	24.37		Medium	Low			
		LS5	6.15	2.19	1.9	7.78	7.78
		MS2	0	3.88	1.86	7.63	15.41
		Low profile	6.36	2.93	1.64	6.73	22.14
		HS5	0	3.38	1.62	6.63	28.77
		Late colonists	1.15	4.25	1.49	6.09	34.86
		Monoraphideae	5.26	2.41	1.37	5.61	40.47
		Centric	2.6	1.16	1.24	5.1	45.57
		Size 2	1.25	3.73	1.19	4.86	50.44
		Biraphideae	6.93	9.34	1.15	4.73	55.16
		High profile	2.81	4.65	1.01	4.13	59.29
		MS5	1.81	2.97	0.96	3.95	63.24
		Colonial	4.09	2.33	0.86	3.52	66.76
		Motile	7.12	8.09	0.64	2.62	69.38
		Size 3	5.63	4.3	0.64	2.61	71.99
		Size 5	6.51	6.26	0.6	2.45	74.44
		MS4	4.47	5	0.59	2.41	76.85
		Intermediate colonists	8.29	7.19	0.58	2.36	79.22
		LS1	1.13	1.04	0.56	2.28	81.5
		MS3	4.83	3.74	0.54	2.23	83.73
		Pioneer colonists	5.21	5.39	0.53	2.19	85.92
		Size 1	1.06	1.31	0.51	2.08	88.01
		HS3	2.81	2.25	0.48	1.97	89.97
		Araphideae	3.19	2.19	0.48	1.97	91.94
Very high & low	19.64		Very high	Low			
		LS5	5.1	2.19	1.37	6.97	6.97
		HS5	2.31	3.38	1.26	6.41	13.39
		Low profile	5.48	2.93	1.2	6.09	19.48
		Monoraphideae	4.52	2.41	0.99	5.06	24.54
		HS4	2.4	0.3	0.97	4.96	29.5
		MS3	2.93	3.74	0.97	4.93	34.44
		MS5	3.08	2.97	0.95	4.84	39.28
		Centric	1.54	1.16	0.9	4.56	43.84
		Late colonists	2.48	4.25	0.83	4.21	48.05
		MS2	2.2	3.88	0.79	4.03	52.09
		High profile	4.4	4.65	0.76	3.87	55.95
		Size 3	4.18	4.3	0.69	3.53	59.49
		Colonial	2.73	2.33	0.69	3.52	63.01
		Motile	7.02	8.09	0.68	3.48	66.49
		Size 2	2.36	3.73	0.64	3.27	69.76
		Size 5	7.22	6.26	0.63	3.22	72.98
		MS4	3.73	5	0.61	3.13	76.11
		Pioneer colonists	5.68	5.39	0.54	2.73	78.84
		Size 1	0.69	1.31	0.51	2.58	81.42
		Intermediate colonists	7.7	7.19	0.47	2.37	83.8

Appendix B9 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Classification guilds	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
High & low	17.03	Biraphideae	8.35	9.34	0.46	2.36	86.16
		LS1	0.67	1.04	0.44	2.26	88.42
		LS3	0.69	0.62	0.42	2.12	90.54
			High	Low			
		HS5	2.11	3.38	1.27	7.47	7.47
		LS5	4.21	2.19	0.96	5.65	13.11
		High profile	3.08	4.65	0.89	5.25	18.36
		Monoraphideae	4.15	2.41	0.85	4.98	23.35
		Low profile	4.73	2.93	0.85	4.97	28.32
		MS2	2.53	3.88	0.69	4.07	32.39
		Araphideae	1.3	2.19	0.67	3.92	36.31
		Colonial	1.44	2.33	0.66	3.89	40.2
		Size 5	6.32	6.26	0.62	3.66	43.86
		MS5	4.04	2.97	0.62	3.66	47.51
		Pioneer colonists	4.42	5.39	0.61	3.56	51.07
		MS4	4.68	5	0.6	3.53	54.6
		Size 2	2.61	3.73	0.58	3.43	58.03
		Size 1	0.32	1.31	0.57	3.35	61.38
		Size 4	5.15	4.89	0.55	3.23	64.62
		Centric	1.53	1.16	0.54	3.16	67.78
		Motile	8.06	8.09	0.54	3.14	70.93
		LS4	1.13	0	0.53	3.09	74.02
		Intermediate colonists	8.16	7.19	0.51	3	77.02
		Late colonists	3.46	4.25	0.49	2.89	79.91
		LS1	0.3	1.04	0.46	2.7	82.61
		MS3	4	3.74	0.46	2.68	85.28
		LS3	0.67	0.62	0.41	2.42	87.71
		Biraphideae	8.53	9.34	0.38	2.24	89.95
		MS1	0	0.76	0.36	2.12	92.07
Medium & none	26.8		Medium	None			
		Late colonists	1.15	5.45	2.17	8.09	8.09
		Low profile	6.36	2.14	2.13	7.94	16.03
		Colonial	4.09	0	2.06	7.68	23.72
		MS2	0	4.08	2.06	7.67	31.39
		LS5	6.15	2.14	2.02	7.54	38.93
		Monoraphideae	5.26	1.96	1.66	6.21	45.14
		Araphideae	3.19	0	1.61	6	51.13
		Biraphideae	6.93	9.8	1.45	5.39	56.52
		Centric	2.6	0.29	1.31	4.88	61.4
		Size 2	1.25	3.75	1.26	4.69	66.09
		Motile	7.12	9.58	1.24	4.61	70.71
		MS5	1.81	3.83	1.02	3.81	74.51
		Intermediate colonists	8.29	6.62	0.84	3.14	77.66
		HS3	2.81	1.39	0.71	2.66	80.32
		HS5	0	1.32	0.67	2.49	82.81
		LS1	1.13	0	0.57	2.13	84.94
		Pioneer colonists	5.21	5.15	0.56	2.1	87.04
		Size 5	6.51	5.77	0.56	2.07	89.12
		Size 1	1.06	0	0.54	2	91.11
Very high & none	23.92		Very high	None			
		Low profile	5.48	2.14	1.65	6.9	6.9
		LS5	5.1	2.14	1.46	6.12	13.02
		Late colonists	2.48	5.45	1.46	6.11	19.13
		MS3	2.93	5.78	1.43	5.97	25.1
		Colonial	2.73	0	1.33	5.57	30.67
		Monoraphideae	4.52	1.96	1.27	5.29	35.96
		Motile	7.02	9.58	1.26	5.26	41.22
		High profile	4.4	1.92	1.22	5.09	46.31
		HS4	2.4	0	1.18	4.91	51.22
		MS5	3.08	3.83	1.13	4.73	55.96

Appendix B9 continued.

<i>T. granifera</i> density	Overall dissimilarity (%)	Classification guilds	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		MS2	2.2	4.08	0.93	3.88	59.84
		HS5	2.31	1.32	0.93	3.87	63.71
		Centric	1.54	0.29	0.79	3.32	67.03
		Araphideae	1.54	0	0.76	3.16	70.19
		Size 3	4.18	5.46	0.74	3.08	73.27
		Size 5	7.22	5.77	0.72	3.01	76.28
		MS4	3.73	5.19	0.72	3.01	79.3
		Biraphideae	8.35	9.8	0.71	2.99	82.28
		Size 2	2.36	3.75	0.68	2.86	85.14
		HS3	2.65	1.39	0.61	2.57	87.71
		Pioneer colonists	5.68	5.15	0.6	2.53	90.24
		High	None				
		Low profile	4.73	2.14	1.29	7.44	7.44
		Monoraphideae	4.15	1.96	1.09	6.32	13.76
		LS5	4.21	2.14	1.03	5.98	19.75
		Late colonists	3.46	5.45	0.98	5.68	25.43
		MS3	4	5.78	0.88	5.11	30.54
		HS5	2.11	1.32	0.83	4.81	35.34
		MS2	2.53	4.08	0.77	4.47	39.82
		Intermediate colonists	8.16	6.62	0.77	4.45	44.27
		Motile	8.06	9.58	0.75	4.34	48.61
		Centric	1.53	0.29	0.71	4.13	52.74
		Colonial	1.44	0	0.7	4.08	56.82
		Araphideae	1.3	0	0.64	3.68	60.5
		Biraphideae	8.53	9.8	0.63	3.65	64.15
		Size 5	6.32	5.77	0.62	3.6	67.75
		Pioneer colonists	4.42	5.15	0.6	3.45	71.2
		MS4	4.68	5.19	0.57	3.31	74.51
		High profile	3.08	1.92	0.57	3.3	77.81
		Size 2	2.61	3.75	0.57	3.28	81.09
		LS4	1.13	0	0.55	3.2	84.29
		Size 4	5.15	4.78	0.53	3.07	87.37
		HS3	2.34	1.39	0.47	2.71	90.08
		Low	None				
		HS5	3.38	1.32	1.44	9.28	9.28
		High profile	4.65	1.92	1.34	8.61	17.9
		Colonial	2.33	0	1.15	7.38	25.27
		Araphideae	2.19	0	1.08	6.91	32.18
		MS3	3.74	5.78	1.01	6.47	38.66
		Motile	8.09	9.58	0.73	4.69	43.34
		Size 1	1.31	0	0.65	4.15	47.49
		Late colonists	4.25	5.45	0.59	3.79	51.28
		Size 3	4.3	5.46	0.57	3.69	54.97
		MS5	2.97	3.83	0.55	3.52	58.49
		Centric	1.16	0.29	0.52	3.37	61.86
		LS1	1.04	0	0.51	3.29	65.15
		Size 5	6.26	5.77	0.5	3.2	68.36
		MS4	5	5.19	0.48	3.07	71.42
		HS3	2.25	1.39	0.42	2.72	74.14
		LS5	2.19	2.14	0.41	2.61	76.76
		Low profile	2.93	2.14	0.39	2.51	79.27
		MS1	0.76	0	0.38	2.41	81.68
		Intermediate colonists	7.19	6.62	0.37	2.35	84.03
		Size 4	4.89	4.78	0.33	2.09	86.12
		HS2	0.65	0	0.32	2.06	88.18
		LS3	0.62	0	0.3	1.95	90.13

Appendix B10: Contribution of various diatom functional groups within group similarities determined through a Similarity percentage analysis (SIMPER). Groups are based on rivers sampled (Phongolo River 2017, Olifants River 2020 and 2021 or Limpopo River 2021) and were determined using Bray Curtis similarity matrix.

Rivers	Overall similarity (%)	Classification guilds	Average abundance	Average similarity	Contribution (%)	Cumulative contribution (%)
Phongolo	80.68	Intermediate colonists	8.22	7.67	9.5	19.86
		Biraphideae	7.69	6.81	8.44	28.3
		Motile	7.59	6.78	8.4	36.7
		Size 3	5.61	5.3	6.57	43.27
		Size 5	6.03	5.06	6.27	49.54
		MS3	5.01	4.58	5.67	55.21
		Low profile	5.34	4.39	5.44	60.66
		LS5	5.11	4.18	5.18	65.84
		Size 4	4.95	4.12	5.11	70.95
		Monoraphideae	4.51	3.78	4.69	75.64
		MS4	4.64	3.72	4.62	80.25
		Pioneer colonists	4.83	3.72	4.61	84.87
		High profile	3.27	2.34	2.91	87.77
		HS3	2.69	2.2	2.72	90.5
Olifants	83.85	Mobile	9.77	9.09	10.84	10.84
		Biraphideae	8.63	7.75	9.24	20.08
		Intermediate colonists	7.87	6.93	8.27	28.35
		Size 5	7.37	6.42	7.65	36.01
		Motile	7.14	6.22	7.42	43.43
		Pioneer colonists	5.2	4.22	5.03	48.46
		Size 4	4.44	3.82	4.56	53.02
		Low profile	4.99	3.77	4.49	57.51
		MS4	4	3.4	4.05	61.57
		LS5	4.52	3.39	4.04	65.61
		High profile	4.39	3.17	3.78	69.38
		Size 3	3.88	3	3.58	72.96
		Monoraphideae	4.2	2.98	3.56	76.52
		HS5	3.92	2.88	3.44	79.96
		Late colonists	3.04	2.65	3.15	83.12
		MS5	3.67	2.21	2.63	85.75
		Size 2	2.67	2.06	2.45	88.2
		HS3	2.49	1.84	2.19	90.39
Limpopo	86.62	Mobile	9.84	9.45	10.91	10.91
		Biraphideae	9.49	9.03	10.42	21.34
		Motile	9.09	8.45	9.76	31.1
		Intermediate colonists	6.71	6.25	7.21	38.31
		Pioneer colonists	5.56	5.18	5.98	44.29
		Size 5	5.74	5.09	5.87	50.16
		MS4	5.26	4.41	5.09	55.26
		Size 3	4.87	4.34	5.02	60.27
		Size 4	5.02	4.34	5.01	65.28
		Late colonists	4.83	4.2	4.84	70.13
		MS3	4.77	4.09	4.72	74.85
		MS2	4.28	3.94	4.54	79.4
		Size 2	3.95	3.71	4.29	83.68
		MS5	3.46	2.75	3.18	86.86
		Low profile	2.67	2.2	2.54	89.4
		High profile	2.98	2.15	2.48	91.88

Appendix B11: Contribution of various diatom functional groups within pairwise group dissimilarities determined through a Similarity percentage analysis (SIMPER). Groups are based on rivers sampled (Phongolo River 2017, Olifants River 2020 and 2021 or Limpopo River 2021) and were determined using Bray Curtis similarity matrix.

Rivers	Overall dissimilarity (%)	Classification guilds	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
			Phongolo	Olifants			
Phongolo & Olifants	20.57	HS5	0	3.92	1.87	9.11	9.11
		Centric	3	0.48	1.32	6.42	15.52
		MS3	5.01	2.62	1.15	5.58	21.11
		Colonial	3.33	2	1.14	5.55	26.66
		MS5	2.26	3.67	1.09	5.31	31.97
		High profile	3.27	4.39	0.84	4.06	36.03
		Size 3	5.61	3.88	0.83	4.04	40.08
		Size 5	6.03	7.37	0.77	3.73	43.8
		Late colonists	2.15	3.04	0.75	3.63	47.43
		Monoraphideae	4.51	4.2	0.73	3.56	50.99
		MS2	1.24	2.42	0.73	3.55	54.54
		LS5	5.11	4.52	0.73	3.54	58.08
		HS4	1.05	0.95	0.72	3.51	61.59
		Pioneer colonists	4.83	5.2	0.7	3.42	65.01
		Low profile	5.34	4.99	0.7	3.42	68.44
		Araphideae	1.99	1.88	0.63	3.04	71.48
		MS4	4.64	4	0.58	2.84	74.32
		Biraphideae	7.69	8.63	0.58	2.8	77.12
		Size 2	1.78	2.67	0.56	2.73	79.85
		Size 1	0.53	0.96	0.48	2.35	82.2
		Size 4	4.95	4.44	0.48	2.34	84.55
		Motile	7.59	7.14	0.48	2.31	86.86
		LS1	0.57	0.73	0.45	2.17	89.03
		HS3	2.69	2.49	0.4	1.92	90.95
			Phongolo	Limpopo			
Phongolo & Limpopo	21.43	LS5	5.11	1.96	1.53	7.15	7.15
		MS2	1.24	4.28	1.47	6.87	14.02
		Late colonists	2.15	4.83	1.34	6.24	20.26
		Low profile	5.34	2.67	1.3	6.05	26.31
		Colonial	3.33	1.45	1.26	5.89	32.2
		Centric	3	1.26	1.14	5.34	37.54
		Monoraphideae	4.51	2.23	1.11	5.17	42.71
		Size 2	1.78	3.95	1.05	4.89	47.6
		Biraphideae	7.69	9.49	0.87	4.07	51.67
		HS5	0	1.71	0.83	3.86	55.53
		Motile	7.59	9.09	0.77	3.59	59.12
		MS5	2.26	3.46	0.75	3.49	62.61
		Intermediate colonists	8.22	6.71	0.73	3.43	66.04
		Araphideae	1.99	1.37	0.72	3.34	69.38
		MS4	4.64	5.26	0.63	2.96	72.33
		Pioneer colonists	4.83	5.56	0.63	2.95	75.28
		High profile	3.27	2.98	0.59	2.77	78.05
		HS4	1.05	0.3	0.57	2.65	80.71
		Size 5	6.03	5.74	0.51	2.39	83.09
		HS3	2.69	1.84	0.49	2.29	85.38
		Size 4	4.95	5.02	0.47	2.22	87.6
		LS1	0.57	0.78	0.47	2.19	89.79
		Size 1	0.53	0.71	0.43	1.99	91.79

Appendix B11 continued.

Rivers	Overall dissimilarity (%)	Classification guilds	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
			Olifants	Limpopo			
Olifants & Limpopo	19.21	LS5	4.52	1.96	1.23	6.4	6.4
		HS5	3.92	1.71	1.21	6.27	12.67
		Low profile	4.99	2.67	1.14	5.93	18.6
		MS3	2.62	4.77	1.05	5.44	24.05
		Monoraphideae	4.2	2.23	0.96	4.98	29.03
		Motile	7.14	9.09	0.93	4.86	33.89
		MS2	2.42	4.28	0.89	4.64	38.53
		Late colonists	3.04	4.83	0.86	4.49	43.02
		High profile	4.39	2.98	0.85	4.41	47.42
		MS5	3.67	3.46	0.83	4.31	51.73
		Size 5	7.37	5.74	0.79	4.1	55.83
		MS4	4	5.26	0.65	3.38	59.21
		Size 2	2.67	3.95	0.61	3.19	62.4
		Centric	0.48	1.26	0.59	3.05	65.44
		Intermediate colonists	7.87	6.71	0.58	3.04	68.48
		Colonial	2	1.45	0.55	2.89	71.37
		Size 3	3.88	4.87	0.54	2.8	74.17
		Araphideae	1.88	1.37	0.53	2.74	76.9
		Pioneer colonists	5.2	5.56	0.5	2.6	79.5
		Size 1	0.96	0.71	0.49	2.56	82.06
		LS1	0.73	0.78	0.49	2.55	84.61
		HS3	2.49	1.84	0.45	2.35	86.96
		Biraphideae	8.63	9.49	0.42	2.18	89.14
		Size 4	4.44	5.02	0.4	2.08	91.22

Appendix C

Appendix C1: *In situ* water quality and water nutrient parameters of individual microcosms measured at time of diatom and macroinvertebrate community sampling.

Microcosm	<i>Tarebia</i> 1	<i>Tarebia</i> 2	Control 1	Control 2	<i>Melanoides</i> 1	<i>Melanoides</i> 2
pH	7.8	7.71	7.62	7.96	7.76	7.64
Temperature	23	22.8	22.7	22.4	22.4	22.2
Conductivity	756	740	674	634	675	675
Total dissolved solids (TDS)	572	528	466	480	462	471
O ₂ %	93.6	70.4	70.1	77.9	77.9	64.3
[O ₂] (mg/L)	11.93	10.3	10.4	11.2	11.36	10.82
NH ₄ (mg/L)	0.12	0.17	0.13	0.12	0.11	0.07
NO ₃ (mg/L)	9.7	10.9	0.8	0.9	5.8	13.4
SO ₄ (mg/L)	33	28	30	28	32	26
Cl (mg/L)	100	89	93	90	89	84
PO ₄ (mg/L)	0.3	0.3	0.3	0.4	0.3	0.5

Appendix C2: List of diatom species sampled from the microcosms on the Phongolo River Floodplain in the Ndumo Game Reserve, KwaZulu-Natal. Control: no snails, *Tarebia*: invasive *Tarebia granifera*, and *Melanoides*: native *Melanoides tuberculata*. CEMFG's = Combined ecological morphological functional groups.

Species	Colonisation strategy	Group	Size class	Life-forms			Ecological guilds			CEMFG's	Microcosms					
				Mobile	Pioneer	Colonial	High profile	Low profile	Motile		Control		Melanoides		Tarebia	
											µ-Count	%	µ-Count	%	µ-Count	%
<i>Amphora veneta</i> Kützing	Intermediate	Biraphideae	5	1	0	0	0	1	0	LS5	0	0	3	1.70	0	0
<i>Bacillaria paradoxa</i> Gmelin	Intermediate	Biraphideae	5	1	0	0	0	0	1	MS5	0	0	0	0	5	3.67
<i>Cocconeis placentula</i> (Ehrenberg) Heinzerling	Intermediate	Monoraphideae	5	1	0	0	0	1	0	LS5	0	0	0	0	2	1.63
<i>Craticula cuspidata</i> (Kützing) DG Mann	Late	Biraphideae	5	1	0	0	0	0	1	MS5	0	0	0	0	2	1.63
<i>Craticula halophila</i> (Grunow) DG Mann	Late	Biraphideae	3	1	0	0	0	0	1	MS3	0	0	0	0	3	2.45
<i>Craticula molestiformis</i> (Hustedt) Lange-Bertalot	Late	Biraphideae	2	1	0	0	0	0	1	MS2	0	0	10	6.46	0	0
Σ- <i>Craticula</i> sp.												0		6.46		4.08
<i>Cyclotella meneghiniana</i> Kützing	Pioneer	Centric	4	0	0	0	0	0	0	-	0	0	0	0	3	2.45
<i>Cyclotella ocellata</i> Pantocsek	Pioneer	Centric	3	0	0	0	0	0	0	-	0	0	0	0	2	1.22
Σ- <i>Cyclotella</i> sp.												0		0		3.67
<i>Eunotia flexuosa</i> Brébisson) Kützing	Intermediate	Raphidioideae	5	1	0	1	1	0	0	HS5	2	2.04	0	0	0	0
<i>Eunotia incisa</i> Gregory	Intermediate	Raphidioideae	4	1	0	1	1	0	0	HS4	12	1.53	0	0	0	0
Σ- <i>Eunotia</i> sp.												3.57		0		0
<i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	Pioneer	Araphideae	5	0	0	1	1	0	0	HS5	23	22.96	3	2.04	23	18.78
<i>Fragilaria capucina</i> Desmazières	Pioneer	Araphideae	2	0	0	1	1	0	0	HS2	0	0	0	0	7	5.71
<i>Fragilaria ulna</i> (Nitzsch) Lange-Bertalot	Pioneer		5	0	0	1	1	0	0	HS5	4	4.08	0	0	0	0
<i>Fragilaria ulna</i> var. <i>acus</i> (Kützing)	Pioneer	Araphideae	3	0	0	1	0	0	0	-	8	8.16	6	3.74	0	0
Σ- <i>Fragilaria</i> sp.												35.20		5.78		24.49
<i>Gomphonema affine</i> Kützing	Intermediate	Biraphideae	4	1	0	0	1	0	0	HS4	2	2.04	9	6.12	2	1.22
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	0	0	4	2.72	0	0
<i>Gomphonema clavatum</i> Ehrenberg	Intermediate	Biraphideae	4	1	0	0	1	0	0	HS4	3	3.06	0	0	1	0.82
<i>Gomphonema insigne</i> Gregory	Intermediate	Biraphideae	5	1	0	0	1	0	0	HS5	3	3.06	0	0	16	12.65

<i>Gomphonema lagenula</i> Kützing	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	0	0	0	0	7	5.71
<i>Gomphonema parvulum</i> (Kützing) Kützing sensu stricto	Intermediate	Biraphideae	3	1	0	0	1	0	0	HS3	0	0	4	2.72	0	0
<i>Gomphonema pseudoagur</i> Krammer	Intermediate	Biraphideae	4	1	0	0	1	0	0	HS4	0	0	0	0	4	3.27
Σ-Gomphonema sp.												8.16		11.56		23.67
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	Pioneer	Biraphideae	3	1	0	0	0	0	0	MS3	2	2.04	0	0	0	0
<i>Navicula radiosa</i> Kützing	Intermediate	Biraphideae	5	1	0	0	0	0	0	MS5	3	3.06	0	0	0	0
<i>Navicula recens</i> Lange-Bertalot	Intermediate	Biraphideae	4	1	0	0	0	0	0	MS4	6	5.61	13	8.50	8	6.12
Σ-Navicula sp.												8.67		8.50		6.12
<i>Nitzschia dissipata</i> (Kützing) Grunow	Intermediate	Biraphideae	4	1	0	0	0	0	0	MS4	0	0	22	14.63	0	0
<i>Nitzschia dissipata</i> var. <i>media</i> (Hantzsch) Grunow	Intermediate	Biraphideae	4	1	0	0	0	0	0	MS4	8	8.16	0	0	14	11.02
<i>Nitzschia etoshensis</i> Cholnoky	Intermediate	Biraphideae	3	1	0	0	0	0	0	MS3	10	9.69	12	7.82	0	0
<i>Nitzschia intermedia</i> Hantzsch	Intermediate	Biraphideae	4	1	0	0	0	0	0	MS4	7	6.63	5	3.06	0	0
<i>Nitzschia linearis</i> (Agardh) W Smith	Intermediate	Biraphideae	5	1	0	0	0	0	0	MS5	6	6.61	16	10.88	18	14.29
<i>Nitzschia littorea</i> Grunow	Intermediate	Biraphideae	3	1	0	0	0	0	0	MS3	0	0	3	2.04	0	0
<i>Nitzschia obtusa</i> W Smith	Intermediate	Biraphideae	5	1	0	0	0	0	0	MS5	0	0	9	6.12	0	0
<i>Nitzschia pura</i> Hustedt	Intermediate	Biraphideae	3	1	0	0	0	0	0	MS3	6	5.61	0	0	0	0
<i>Nitzschia recta</i> Hantzsch	Intermediate	Biraphideae	4	1	0	0	0	0	0	MS4	5	4.59	21	14.29	0	0
<i>Nitzschia sigma</i> (Kützing) W Smith	Intermediate	Biraphideae	5	1	0	0	0	0	0	MS5	0	0	0	0	6	4.90
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot	Intermediate	Biraphideae	5	1	0	0	0	0	0	MS5	2	2.04	0	0	0	0
Σ-Nitzschia sp.												43.33		58.84		30.21
<i>Tryblionella apiculata</i> Gregory	Late	Biraphideae	4	1	0	0	0	0	0	MS4	0	0	11	7.14	4	2.86
Diatom Community Characterisation %											Control		Melanoides		Tarebia	
Colonisation strategy										Pioneer colonists	37.24		5.78		27.76	
										Intermediate colonists	62.76		80.61		65.31	
										Late colonists	0		13.61		6.94	
Group										Monoraphideae	0		0		1.63	
										Biraphideae	61.22		94.22		70.61	
										Centric	0		0		3.27	
										Araphideae	35.20		5.78		24.49	
										Raphidioideae	3.57		0		0	
Size class										Size 1	0		0		0	
										Size 2	0		6.46		5.71	
										Size 3	25.51		19.05		9.39	
										Size 4	31.63		53.74		27.35	
										Size 5	42.86		20.75		57.55	
Life-forms										Mobile	62.56		94.22		74.68	

	Pioneer	0	0	0
	Colonial	37.44	5.78	25.32
Ecological guilds	High profile	42.22	14.13	49.79
	Low profile	0	1.77	1.69
	Motile	57.78	84.10	48.52
CEMFG's	HS1	0	0	0
	HS2	0	0	5.91
	HS3	0	5.65	5.91
	HS4	7.22	6.36	5.49
	HS5	35	2.12	32.49
	LS1	0	0	0
	LS2	0	0	0
	LS3	0	0	0
	LS4	0	0	0
	LS5	0	1.77	1.69
	MS1	0	0	0
	MS2	0	6.71	0
	MS3	18.89	10.25	2.53
	MS4	27.22	49.47	20.68
	MS5	11.67	17.67	25.32

Appendix C3: Contribution of various diatom species within group similarities determined through a Similarity percentage analysis (SIMPER). Groups are based on snail presence (none, native, invasive) sampled from microcosms on the Phongolo River Floodplain in 2017 and were determined using Bray Curtis similarity matrix.

Snails	Overall similarity (%)	Species	Average abundance	Average similarity	Contribution (%)	Cumulative contribution (%)
None	24.52	<i>Nitzschia etoshensis</i>	3.08	9.57	39.03	39.03
		<i>Fragilaria biceps</i>	4.35	7.81	31.87	70.91
		<i>Navicula recens</i>	2.34	7.13	29.09	100
Native	43.64	<i>Nitzschia linearis</i>	4	10.36	23.74	23.74
		<i>Nitzschia recta</i>	4.52	9.69	22.2	45.94
		<i>Navicula recens</i>	3.53	8.97	20.56	66.5
		<i>Gomphonema affine</i>	3	7.77	17.8	84.3
		<i>Craticula molestiformis</i>	3.05	6.85	15.7	100
Invasive	17.84	<i>Fragilaria biceps</i>	4.7	11.62	65.17	65.17
		<i>Navicula recens</i>	2.66	6.21	34.83	100

Appendix C4: Contribution of various diatom species within pairwise group dissimilarities determined through a Similarity percentage analysis (SIMPER). Groups are based on snail presence (none, native, invasive) sampled from microcosms on the Phongolo River Floodplain in 2017 and were determined using Bray Curtis similarity matrix.

Snails	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
			None	Native			
None & native	67.20	<i>Nitzschia recta</i>	1.5	4.52	4.59	6.82	6.82
		<i>Craticula molestiformis</i>	0	3.05	4.36	6.49	13.32
		<i>Nitzschia dissipata</i>	0	3.28	4.22	6.29	19.6
		<i>Fragilaria biceps</i>	4.35	1.22	4.22	6.28	25.88
		<i>Nitzschia etoshensis</i>	3.08	2.4	3.37	5.01	30.89
		<i>Nitzschia linearis</i>	1.66	4	3.3	4.91	35.8
		<i>Nitzschia dissipata</i> var. <i>media</i>	2	0	3.02	4.49	40.28
		<i>Gomphonema affine</i>	1	3	2.96	4.4	44.69
		<i>Tryblionella apiculata</i>	0	2.29	2.95	4.39	49.08
		<i>Fragilaria ulna</i> var. <i>acus</i>	2	1.66	2.86	4.25	53.33
		<i>Nitzschia ubtusa</i>	0	2.12	2.73	4.07	57.4
		<i>Nitzschia intermedia</i>	1.8	1.5	2.65	3.94	61.34
		<i>Nitzschia pura</i>	1.66	0	2.5	3.72	65.06
		<i>Gomphonema angustatum</i>	0	1.41	2.28	3.39	68.45
		<i>Fragilaria ulna</i>	1.41	0	1.97	2.93	71.38
		<i>Gomphonema insigne</i>	1.22	0	1.85	2.75	74.13
		<i>Gomphonema parvulum</i>	0	1.41	1.82	2.71	76.84
		<i>Amphora veneta</i>	0	1.12	1.8	2.68	79.52
		<i>Navicula recens</i>	2.34	3.53	1.73	2.58	82.1
		<i>Gomphonema clavatum</i>	1.22	0	1.7	2.54	84.64
		<i>Navicula radiosa</i>	1.22	0	1.7	2.54	87.17
		<i>Nitzschia littorea</i>	0	1.22	1.58	2.35	89.52
		<i>Eunotia flexuosa</i>	1	0	1.51	2.24	91.76
None & invasive	71.56		None	Invasive			
		<i>Nitzschia etoshensis</i>	3.08	0	4.87	6.8	6.8
		<i>Nitzschia linearis</i>	1.66	2.96	4.58	6.4	13.2
		<i>Gomphonema insigne</i>	1.22	2.78	4.52	6.32	19.52
		<i>Nitzschia dissipata</i> var. <i>media</i>	2	2.6	4.14	5.79	25.32
		<i>Gomphonema lagenula</i>	0	1.87	3.11	4.35	29.67
		<i>Fragilaria ulna</i> var. <i>acus</i>	2	0	3.02	4.23	33.89
		<i>Fragilaria biceps</i>	4.35	4.7	3.02	4.22	38.11
		<i>Nitzschia intermedia</i>	1.8	0	2.98	4.16	42.27
		<i>Fragilaria capucina</i>	0	1.87	2.8	3.92	46.19
		<i>Nitzschia pura</i>	1.66	0	2.74	3.82	50.01
		<i>Nitzschia sigma</i>	0	1.73	2.59	3.63	53.64
		<i>Gomphonema pseudoagur</i>	0	1.41	2.35	3.29	56.93

Appendix C4 continued.

Snails	Overall dissimilarity (%)	Species	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
		<i>Nitzschia recta</i>	1.5	0	2.27	3.17	60.1
		<i>Bacillaria paradoxa</i>	0	1.5	2.25	3.14	63.24
		<i>Fragilaria ulna</i>	1.41	0	2.14	2.99	66.22
		<i>Craticula halophila</i>	0	1.22	2.04	2.85	69.07
		<i>Tryblionella apiculata</i>	0	1.32	1.98	2.77	71.84
		<i>Gomphonema clavatum</i>	1.22	0.71	1.9	2.65	74.49
		<i>Navicula radiosa</i>	1.22	0	1.85	2.59	77.08
		<i>Cyclotella meneghiniana</i>	0	1.12	1.67	2.34	79.42
		<i>Cocconeis placentula</i>	0	1	1.66	2.33	81.75
		<i>Eunotia flexuosa</i>	1	0	1.65	2.31	84.05
		<i>Nitzschia umbonata</i>	1	0	1.65	2.31	86.36
		<i>Gomphonema affine</i>	1	0.87	1.57	2.19	88.55
		<i>Amphora</i> sp.	1	0	1.51	2.11	90.66
			Native	Invasive			
		<i>Nitzschia recta</i>	4.52	0	6.59	8.35	8.35
		<i>Fragilaria biceps</i>	1.22	4.7	4.72	5.98	14.33
		<i>Craticula molestiformis</i>	3.05	0	4.31	5.46	19.79
		<i>Nitzschia linearis</i>	4	2.96	4.31	5.46	25.25
		<i>Gomphonema insigne</i>	0	2.78	4.18	5.3	30.54
		<i>Nitzschia dissipata</i>	3.28	0	4.18	5.29	35.84
		<i>Nitzschia dissipata</i> var. <i>media</i>	0	2.6	3.9	4.94	40.78
		<i>Nitzschia etoshensis</i>	2.4	0	3.81	4.83	45.61
		<i>Gomphonema affine</i>	3	0.87	3.12	3.95	49.56
		<i>Tryblionella apiculata</i>	2.29	1.32	3.11	3.94	53.5
		<i>Gomphonema lagenula</i>	0	1.87	2.81	3.56	57.06
		<i>Nitzschia ubtusa</i>	2.12	0	2.7	3.43	60.48
		<i>Fragilaria capucina</i>	0	1.87	2.55	3.23	63.71
		<i>Nitzschia sigma</i>	0	1.73	2.36	2.99	66.71
		<i>Gomphonema angustatum</i>	1.41	0	2.25	2.85	69.55
		<i>Gomphonema pseudoagur</i>	0	1.41	2.12	2.69	72.24
		<i>Fragilaria ulna</i> var. <i>acus</i>	1.66	0	2.11	2.68	74.92
		<i>Bacillaria paradoxa</i>	0	1.5	2.05	2.59	77.51
		<i>Nitzschia intermedia</i>	1.5	0	1.91	2.42	79.93
		<i>Craticula halophila</i>	0	1.22	1.84	2.33	82.27
		<i>Gomphonema parvulum</i>	1.41	0	1.8	2.28	84.55
		<i>Amphora veneta</i>	1.12	0	1.78	2.25	86.8
		<i>Nitzschia littorea</i>	1.22	0	1.56	1.98	88.78
		<i>Cyclotella meneghiniana</i>	0	1.12	1.52	1.93	90.71
Native & invasive	78.96						

Appendix C5: Contribution of various diatom functional groups within group similarities determined through a Similarity percentage analysis (SIMPER). Groups are based on snail presence (none, native, invasive) sampled from microcosms on the Phongolo River Floodplain in 2017 and were determined using Bray Curtis similarity matrix.

Snails	Overall similarity (%)	Classification guilds	Average abundance	Average similarity	Contribution (%)	Cumulative contribution (%)
None	76.28	Mobile	7.94	8.29	10.87	10.87
		Intermediate colonists	7.95	8.04	10.54	21.4
		Motile	7.52	7.73	10.13	31.54
		Size 5	6.47	7.72	10.12	41.66
		Size 4	5.67	6.37	8.35	50
		Size 3	5.02	6.2	8.13	58.13
		High profile	6.15	5.54	7.27	65.4
		HS5	5.69	5.54	7.27	72.67
		MS3	4.34	4.96	6.5	79.17
		MS4	5.12	4.96	6.5	85.67
		Colonial	5.49	4.28	5.62	91.29
Native	87.44	Mobile	9.71	11.61	13.27	13.27
		Motile	9.09	10.42	11.92	25.19
		Intermediate colonists	9.05	10.4	11.9	37.08
		Size 4	7.18	7.9	9.03	46.12
		MS4	6.82	7.15	8.18	54.3
		Size 5	4.6	5.22	5.97	60.27
		Size 3	4.46	4.48	5.12	65.39
		MS5	4.11	4.47	5.11	70.5
		High profile	3.83	3.81	4.36	74.85
		Late colonists	3.38	2.96	3.38	78.23
		Size 2	2.53	2.96	3.38	81.61
		MS2	2.57	2.96	3.38	84.99
		HS4	2.56	2.77	3.17	88.16
		Pioneer colonists	2.38	2.74	3.13	91.29
Invasive	71.61	Mobile	8.63	9.56	13.36	13.36
		Intermediate colonists	8.08	8.57	11.97	25.32
		Size 5	7.47	7.91	11.05	36.37
		High profile	7.05	7.84	10.94	47.31
		Motile	6.91	7.5	10.47	57.78
		HS5	5.69	6.21	8.67	66.45
		Size 4	5.17	4.87	6.8	73.25
		Pioneer colonists	4.97	4.23	5.9	79.15
		Colonial	4.81	4.23	5.9	85.06
		MS4	4.38	3.64	5.08	90.14

Appendix C6: Contribution of various diatom functional groups within pairwise group dissimilarities determined through a Similarity percentage analysis (SIMPER). Groups are based on snail presence (none, native, invasive) sampled from microcosms on the Phongolo River Floodplain in 2017 and were determined using Bray Curtis similarity matrix.

Snails	Overall dissimilarity (%)	Classification guilds	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
			None	Native			
None & native	25.62	HS5	5.69	1.14	2.83	11.03	11.03
		Late colonists	0	3.38	2.11	8.24	19.26
		Colonial	5.49	2.38	1.91	7.44	26.7
		Pioneer colonists	5.22	2.38	1.74	6.78	33.48
		MS2	0	2.57	1.6	6.23	39.71
		Size 2	0	2.53	1.57	6.13	45.83
		HS3	0	2.41	1.49	5.82	51.66
		High profile	6.15	3.83	1.48	5.79	57.45
		MS4	5.12	6.82	1.18	4.62	62.06
		HS4	1.86	2.56	1.16	4.53	66.59
		Size 5	6.47	4.6	1.16	4.53	71.12
		Mobile	7.94	9.71	1.09	4.24	75.35
		Motile	7.52	9.09	1.07	4.16	79.51
		Intermediate colonists	7.95	9.05	1.04	4.07	83.58
		Size 4	5.67	7.18	0.94	3.67	87.25
		MS3	4.34	3.18	0.92	3.61	90.86
None & invasive	23.10		None	Invasive			
		MS5	3.35	3.51	2.19	9.47	9.47
		MS3	4.34	1.14	2	8.66	18.14
		Late colonists	0	2.6	1.62	7.01	25.15
		Pioneer colonists	5.22	4.97	1.61	6.96	32.11
		Size 3	5.02	2.84	1.36	5.88	37.99
		Colonial	5.49	4.81	1.34	5.79	43.78
		HS4	1.86	2.33	1.16	5.04	48.83
		High profile	6.15	7.05	1.16	5	53.83
		HS3	0	1.74	1.09	4.71	58.54
		HS2	0	1.69	1.06	4.58	63.12
		Intermediate colonists	7.95	8.08	1.06	4.58	67.69
		MS4	5.12	4.38	1.06	4.58	72.27
		Size 2	0	1.64	1.02	4.44	76.7
		Motile	7.52	6.91	0.94	4.09	80.79
		Mobile	7.94	8.63	0.92	3.99	84.79
		HS5	5.69	5.69	0.86	3.72	88.51
		Size 5	6.47	7.47	0.75	3.27	91.78
Native & invasive	26.10		Native	Invasive			
		HS5	1.14	5.69	2.75	10.55	10.55
		MS5	4.11	3.51	2.11	8.09	18.65
		High profile	3.83	7.05	1.95	7.47	26.12
		Size 5	4.6	7.47	1.73	6.64	32.75
		Pioneer colonists	2.38	4.97	1.56	5.97	38.72
		MS2	2.57	0	1.55	5.94	44.66
		MS4	6.82	4.38	1.49	5.7	50.36
		Colonial	2.38	4.81	1.47	5.62	55.97
		MS3	3.18	1.14	1.33	5.11	61.08
		Motile	9.09	6.91	1.32	5.06	66.14
		Size 4	7.18	5.17	1.22	4.67	70.81
		Size 3	4.46	2.84	1.1	4.22	75.03
		HS3	2.41	1.74	1.05	4.03	79.06
		HS2	0	1.69	1.02	3.92	82.97
		Size 2	2.53	1.64	0.99	3.79	86.76
		Intermediate colonists	9.05	8.08	0.71	2.7	89.46
		Mobile	9.71	8.63	0.65	2.49	91.95

Appendix D

Appendix D1: Summary data for trematodes transmitted by *Tarebia granifera*.

Trematodes species	Family	1st Intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
<i>Acanthatrium histaense</i> (Koga, 1953)	Lecithodendriidae	<i>Tarebia granifera</i>	Insects, Amphibia	Thailand (Veeravechsukij <i>et al.</i> , 2018a; Dechruksa <i>et al.</i> , 2007).
<i>Acanthotrema tridactyla</i> (Martin & Kuntz, 1955)	Heterophyidae	<i>Tarebia granifera</i>	Fish, Birds, Mammals, Humans	Thailand (Veeravechsukij <i>et al.</i> , 2018a).
<i>Alaria mustelae</i> (Bosma, 1931)	Diplostomidae	<i>Tarebia granifera</i>	Amphibia, Mammals	Thailand (Veeravechsukij <i>et al.</i> , 2018a; Bosma, 1934).
<i>Cardicola alseae</i> (Meade & Pratt, 1965)	Sanguinicolidae	<i>Tarebia granifera</i>	Fish	Thailand (Veeravechsukij <i>et al.</i> , 2018a; Ukong <i>et al.</i> , 2007).
<i>Centrocestus formosanus</i> (Nishigori, 1924)	Heterophyidae	<i>Tarebia granifera</i>	Fish, Birds, Mammals, Humans	Thailand (Veeravechsukij <i>et al.</i> , 2018a; Dechruksa <i>et al.</i> , 2007).
<i>Diorchitrema formosanum</i> (Katsuta, 1932)	Heterophyidae	<i>Tarebia granifera</i>	Fish, Mammals	Taiwan (Abbott, 1952).
<i>Haematoloechus similis</i> (Looss, 1899)	Haematoloechidae	<i>Tarebia granifera</i>	Amphibia	Thailand (Dechruksa <i>et al.</i> , 2007; Grabda, 1960).
<i>Haplorchis pumilio</i> (Looss, 1896)	Heterophyidae	<i>Tarebia granifera</i>	fish, Birds, Mammals, Humans	Thailand (Veeravechsukij <i>et al.</i> , 2018a).
<i>Haplorchis</i> sp.	Heterophyidae	<i>Tarebia granifera</i>	Fish, Birds, Mammals, Humans	Laos (Giboda <i>et al.</i> , 1991b).
<i>Haplorchis taichui</i> (Nishigori, 1924)	Heterophyidae	<i>Tarebia granifera</i>	Fish, Birds, Mammals, Humans	Thailand (Veeravechsukij <i>et al.</i> , 2018a; Le <i>et al.</i> , 2017; Ditrich <i>et al.</i> , 1992; Chontanarith & Wongsawad, 2010; Chontanarith & Wongsawad, 2013).

Appendix D1 continued.

Trematodes species	Family	1st Intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
<i>Loxogenes liberum</i> (Seno, 1907)	Lecithodendriidae	<i>Tarebia granifera</i>	Insects, Amphibia	Thailand (Veeravechsukij <i>et al.</i> , 2018a).
<i>Loxogenoides bicolor</i> (Kaw, 1945)	Lecithodendriidae	<i>Tarebia granifera</i>	Insects, Amphibia	Thailand (Veeravechsukij <i>et al.</i> , 2018a; Dechruksa <i>et al.</i> , 2007).
<i>Maritreminoides caridinae</i> (Yamaguti & Nisimura, 1944)	Microphallidae	<i>Tarebia granifera</i>	Crustacea, Birds	Thailand (Veeravechsukij <i>et al.</i> , 2018a).
<i>Maritreminoides obstipus</i> (Van Cleave & Mueller, 1932)	Microphallidae	<i>Tarebia granifera</i>	Crustacea, Birds	Thailand (Veeravechsukij <i>et al.</i> , 2018a).
<i>Metagonimus yokogawai</i> (Katsurada, 1912)	Heterophyidae	<i>Tarebia granifera</i>	Fish, Mammals, Humans	Japan, Korea, Formosa, Taiwan, India (Abbott, 1952).
<i>Philophthalmus gralli</i> (Mathis & Léger, 1910)	Philophthalmidae	<i>Tarebia granifera</i>	Birds, Mammals, Humans	Thailand (Veeravechsukij <i>et al.</i> , 2018a), United States (Heneberg <i>et al.</i> , 2014; Tolley-Jordan & Owen, 2008).
<i>Philophthalmus</i> sp.	Philophthalmidae	<i>Tarebia granifera</i>	Birds, Mammals, Humans	Jamaica (McKoy <i>et al.</i> , 2011).
<i>Transversotrema laruei</i> (Velasquez, 1958)	Transversotrematidae	<i>Tarebia granifera</i>	Fish	Thailand (Veeravechsukij <i>et al.</i> , 2018a).
<i>Notocotylus</i> sp. (Diesing, 1839)	Notocotylidae	<i>Tarebia granifera</i>	?	Jamaica (McKoy <i>et al.</i> , 2011).

Appendix D2: Summary data for trematodes transmitted by *Melanoides tuberculata*. Data were adapted from Pinto and de Melo (2011).

Trematodes species	Family	1st Intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
<i>Acanthatrium histaense</i> (Koga, 1953)	Lecithodendriidae	<i>Melanoides tuberculata</i>	Insecta, Amphibia	Thailand (Krailas <i>et al.</i> , 2014).
<i>Acanthatrium</i> sp.	Lecithodendriidae	<i>Melanoides tuberculata</i>	Insecta, Amphibia	Vietnam (Besprozvannykh <i>et al.</i> , 2013).
<i>Acanthostomum burminis</i> (Bhalerao, 1926)	Cryptogonimidae	<i>Melanoides tuberculata</i>	Fish, Amphibia	India (Madhavi <i>et al.</i> , 1997; Roopa & Janardanan, 1998).
<i>Acanthotrema tridactyla</i> (Martin & Kuntz, 1955)	Heterophyidae	<i>Melanoides tuberculata</i>	Fish, Birds, Mammals	Thailand (Krailas <i>et al.</i> , 2014).
<i>Alaria mustelae</i> (Bosma, 1931)	Diplostomidae	<i>Melanoides tuberculata</i>	Amphibia, Mammals	Thailand (Krailas <i>et al.</i> , 2014).
<i>Apatemon gracilis</i> (Szidat, 1928)	Strigeidae	<i>Melanoides tuberculata</i>	Fish	Thailand (Krailas <i>et al.</i> , 2014).
<i>Calicophoron microbothrium</i> (Fischöder, 1901)	Paramphistomidae	<i>Melanoides tuberculata</i>	Mammals	Zimbabwe (Chingwena <i>et al.</i> , 2002)
<i>Cardicola alseae</i> (Meade & Pratt, 1965)	Sanguinicolidae	<i>Melanoides tuberculata</i>	Fish	Thailand (Krailas <i>et al.</i> , 2014).
<i>Centrocestus caninus</i> (Leiper, 1913)	Heterophyidae	<i>Melanoides tuberculata</i>	Mammals	Taiwan (Waikagul <i>et al.</i> , 1990).
<i>Centrocestus formosanus</i> (Nishigori, 1924)	Heterophyidae	<i>Melanoides tuberculata</i>	Fish, Birds, Mammals	China (Chen, 1948; Chao <i>et al.</i> , 1993), Japan (Yanohara, 1985; Yanohara <i>et al.</i> , 1987), Mexico (Arizmendi-Espinosa, 1992; Amaya-Huerta & Almeyda-Artigas, 1994; Salgado-Maldonado <i>et al.</i> , 1995; Scholz & Salgado-Maldonado, 2000; Scholz <i>et al.</i> , 2000; Ortega <i>et al.</i> , 2009), Malaysia (Bayssade-Dufour <i>et al.</i> , 1982), Taiwan (Lo & Lee, 1996a, 1996b), India (Madhavi <i>et al.</i> , 1997), United States (Mitchell <i>et al.</i> , 2002, 2005; Tolley-Jordan & Owen, 2008; Huston, 2014),

Appendix D2 continued.

Trematodes species	Family	1st Intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
				Venezuela (Hernández <i>et al.</i> , 2003), Iran (Farahnak <i>et al.</i> , 2005), Colombia (Velásquez <i>et al.</i> , 2006; Vergara & Velásquez, 2009), Brazil (Pinto & de Melo, 2010a; Pulido-Murillo <i>et al.</i> , 2018; Ciccheto <i>et al.</i> , 2021), Thailand (Krailas <i>et al.</i> , 2014), Peru (Pulido-Murillo <i>et al.</i> , 2018).
<i>Centrocestus unequiorchalis</i> (Saad, 1994)	Heterophyidae	<i>Melanoides tuberculata</i>	Birds, Mammals	Egypt (Saad, 1994).
<i>Cloacitrema philippinum</i> (Velasquez, 1969)	Philophthalmidae	<i>Melanoides tuberculata</i>	Birds	Thailand (Krailas <i>et al.</i> , 2014).
<i>Clonorchis sinensis</i> (Cobbold, 1875)	Opisthorchiidae	<i>Melanoides tuberculata</i>	Mammals	China (Galliard, 1938; Lun <i>et al.</i> , 2005), Vietnam (Kino <i>et al.</i> , 1998), unspeciunspecified Asian country (Chai <i>et al.</i> , 2005).
<i>Echinochasmus bagulai</i> (Verma, 1935)	Echinostomatidae	<i>Melanoides tuberculata</i>	Birds	India (Dhanumkumari <i>et al.</i> , 1991a).
<i>Echinochasmus japonicus</i> (Tanabe, 1926)	Echinostomatidae	<i>Melanoides tuberculata</i>	Fish, Birds, Mammals	China (Cheng & Fang, 1989).
<i>Echinochasmus milvi</i> (Yamaguti, 1939)	Echinostomatidae	<i>Melanoides tuberculata</i>	Birds and Mammals	Iran (Farahnak <i>et al.</i> , 2005).
<i>Echinochasmus pelecani</i> (Johnston & Simpson, 1944)	Echinostomatidae	<i>Melanoides tuberculata</i>	Birds	Thailand (Krailas <i>et al.</i> , 2014).
<i>Eumegacetes artamii</i> (Mehra, 1935)	Eumegacetidae	<i>Melanoides tuberculata</i>	Birds	India (Kumari & Madhavi, 1994; Madhavi <i>et al.</i> , 1997).
<i>Eumegacetes spinosus</i> (Fahmy, Khalifa & Abdel-Rahman, 1981)	Eumegacetidae	<i>Melanoides tuberculata</i>	Birds	Egypt (Sakla & Khalifa, 1983).
<i>Gastrodiscus aegyptiacus</i> (Cobbold, 1876)	Gastrodiscidae	<i>Melanoides tuberculata</i>	Mammals	Zimbabwe (Mukaratirwa <i>et al.</i> , 2004).
<i>Gastrothylax crumenifer</i> (Creplin, 1847)	Paramphistomidae	<i>Melanoides tuberculata</i>	Mammals	Thailand (Krailas <i>et al.</i> , 2014).

Appendix D2 continued.

Trematodes species	Family	1st Intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
<i>Gigantobilharzia</i> sp.	Schistosomatidae	<i>Melanoides tuberculata</i>	Birds	Egypt (Fahmy <i>et al.</i> , 1976), United Arab Emirates (Schuster <i>et al.</i> , 2014).
<i>Grysoma indica</i> (Umadevi & Madhavi, 1995)	Psilostomidae	<i>Melanoides tuberculata</i>	Birds	India (Umadevi & Madhavi, 1995; Madhavi <i>et al.</i> , 1997).
<i>Haematoloechus similis</i> (Looss, 1899)	Haematoloechidae	<i>Melanoides tuberculata</i>	Amphibia	Thailand (Krailas <i>et al.</i> , 2014).
<i>Haplorchis pleurolophocerca</i> (Sonsino, 1896)	Heterophyidae	<i>Melanoides tuberculata</i>	Birds, Mammals	Egypt (Faust & Nishigori, 1926; Khalil, 1932).
<i>Haplorchis pumilio</i> (Looss, 1896)	Heterophyidae	<i>Melanoides tuberculata</i>	Fish, Birds, Mammals	China (Shen, 1959; Chao <i>et al.</i> , 1993; Wang <i>et al.</i> , 2002), Malaysia (Ow-Yang & Yen, 1975), Egypt (Khalil, 1932; Khalifa <i>et al.</i> , 1977; Saad & Abed, 1995), Kenya (Sommerville, 1982b), India (Dhanumkumari <i>et al.</i> , 1991b; Madhavi <i>et al.</i> , 1997; Umadevi & Madhavi, 2006; Dechruksa <i>et al.</i> , 2007), Taiwan (Lo & Lee, 1996a, 1996b), Mexico (Scholz <i>et al.</i> , 2000; Scholz <i>et al.</i> , 2001), Iran (Farahnak <i>et al.</i> , 2005), Thailand (Dechruksa <i>et al.</i> , 2007; Krailas <i>et al.</i> , 2011, 2014), United States (Tolley-Jordan & Owen, 2008; Huston, 2014), Venezuela (Díaz <i>et al.</i> , 2008), Vietnam (Skov <i>et al.</i> , 2009; Van <i>et al.</i> , 2009), Brazil (Lopes <i>et al.</i> , 2020), Peru (Pulido-Murillo <i>et al.</i> , 2018).

Appendix D2 continued.

Trematodes species	Family	1st Intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
<i>Haplorchis</i> sp.	Heterophyidae	<i>Melanoides tuberculata</i>	Fish	United States (Harvey <i>et al.</i> , 2005), Zimbabwe (Schols, 2019), Vietnam (Besprozvannykh <i>et al.</i> , 2013).
<i>Haplorchis taichui</i> (Nishigori, 1924)	Heterophyidae	<i>Melanoides tuberculata</i>	Fish, Birds, Mammals	India (Dhanumkumari <i>et al.</i> , 1991b; Madhavi <i>et al.</i> , 1997), Iran (Farahnak <i>et al.</i> , 2005), Thailand (Chuboon & Wongsawad, 2009; Chontanarith & Wongsawad, 2010; Krailas <i>et al.</i> , 2011, 2014).
<i>Haplorchis yokogawai</i> (Katsuta, 1932)	Heterophyidae	<i>Melanoides tuberculata</i>	Mammals	Thailand (Manning <i>et al.</i> , 1971), (Chai <i>et al.</i> , 2005).
<i>Haplorchoides cahirinus</i> (Looss, 1896)	Heterophyidae	<i>Melanoides tuberculata</i>	Fish	Egypt (El-Naffar, 1980).
<i>Haplorchoides mehrai</i> (Pande & Shunkla, 1977)	Heterophyidae	<i>Melanoides tuberculata</i>	Fish	India (Shameem & Madhavi, 1988; Madhavi <i>et al.</i> , 1997).
<i>Heterophyes heterophyes</i> (Siebold, 1852)	Heterophyidae	<i>Melanoides tuberculata</i>	Mammals	(Malek, 1980; Plotnikov, 2003).
Heterophyidae sp.	Heterophyidae	<i>Melanoides tuberculata</i>	?	Brazil (Bogéa <i>et al.</i> , 2005).
Lecithodendriidae sp.	Lecithodendriidae	<i>Melanoides tuberculata</i>	Amphibia, Birds	Zimbabwe (Schols, 2019), Brazil (Lopes <i>et al.</i> , 2021).
<i>Loxogenoides bicolor</i> (Kaw, 1945)	Lecithodendriidae	<i>Melanoides tuberculata</i>	Insecta, Amphibia	Iran (Farahnak <i>et al.</i> , 2005), Thailand (Dechruksa <i>et al.</i> , 2007; Ukong <i>et al.</i> , 2007; Krailas <i>et al.</i> , 2014).
<i>Mesostephanus appendiculatus</i> (Ciurea, 1916)	Cyathocotylidae	<i>Melanoides tuberculata</i>	Fish, Mammals	Thailand (Krailas <i>et al.</i> , 2014).
<i>Mesostephanus haliasturis</i> (Tubangui & Masilungan, 1941)	Cyathocotylidae	<i>Melanoides tuberculata</i>	Birds	Australia (Barker & Cribb, 1993).
<i>Microparyphium</i> sp.	Echinostomatidae	<i>Melanoides tuberculata</i>	Fish	Vietnam (Besprozvannykh <i>et al.</i> , 2013).

Appendix D2 continued.

Trematodes species	Family	1st Intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
<i>Neopronocephalus triangularis</i> (Mehra, 1932)	Pronocephalidae	<i>Melanoides tuberculata</i>	Reptiles	India (Thapar, 1968).
<i>Notocotylus mamii</i> (Hsu, 1954)	Notocotylidae	<i>Melanoides tuberculata</i>	Mammals	China (Hsu, 1957).
<i>Orthetrotrema monostomum</i> (Macy & Basch, 1972)	Eumegacetidae	<i>Melanoides tuberculata</i>	Birds	India (Madhavi & Swamakumari, 1995; Madhavi <i>et al.</i> , 1997).
<i>Paragonimus westermani</i> (Kerbert, 1878)	Paragonimidae	<i>Melanoides tuberculata</i>	Mammals	Taiwan (Nakagawa, 1917), China (Blair <i>et al.</i> , 1999), unspecified Asian country (Davis <i>et al.</i> , 1994).
<i>Paralecithodendrium pyramidium</i> (Looss, 1896)	Lecithodendriidae	<i>Melanoides tuberculata</i>	Mammals	Egypt (Azim, 1936).
<i>Paralecithodendrium</i> sp.	Lecithodendriidae	<i>Melanoides tuberculata</i>	Mammals	Vietnam (Besprozvannykh <i>et al.</i> , 2013).
<i>Paramonostomum aegyptiacus</i> (Khalifa & El-Naffar, 1978)	Notocotylidae	<i>Melanoides tuberculata</i>	Mammals	Egypt (Khalifa & El-Naffar, 1978).
<i>Philophthalmus palpebrarum</i> (Looss, 1899)	Philophthalmidae	<i>Melanoides tuberculata</i>	Birds	Egypt (Ayoub <i>et al.</i> , 2020).
<i>Philophthalmus distomatosa</i> (Looss, 1896)	Philophthalmidae	<i>Melanoides tuberculata</i>	Birds, Mammals	Israel (Radev <i>et al.</i> , 2000).
<i>Philophthalmus gralli</i> (Mathis & Leger, 1910)	Philophthalmidae	<i>Melanoides tuberculata</i>	Birds, Mammals	United States (Murray & Haines, 1969; Nollen & Murray, 1978; Tolley-Jordan & Owen, 2008; Church <i>et al.</i> , 2013), China (Zhongzhang <i>et al.</i> , 1980), Jordan (Ismail & Issa, 1987), India (Rao & Rao, 1981; Karim, 1982; Saxena, 1984), United Arab Emirates (Ismail & Arif, 1992), Saudi Arabia (Kalantan <i>et al.</i> , 1997), Mexico (Scholz <i>et al.</i> , 2000), Venezuela (Díaz <i>et al.</i> , 2002), Zimbabwe (Mukaratirwa <i>et al.</i> , 2005),

Appendix D2 continued.

Trematodes species	Family	1st Intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
				Brazil (Pinto & de Melo, 2010b), Costa Rica (Rojas <i>et al.</i> , 2013).
<i>Philophthalmus nocturnus</i> (Looss, 1907)	Philophthalmidae	<i>Melanoides tuberculata</i>	Birds	India (Madhavi <i>et al.</i> , 1997).
<i>Philophthalmus</i> sp.	Philophthalmidae	<i>Melanoides tuberculata</i>	Birds	Thailand (Krailas <i>et al.</i> , 2014), United Arab Emirates (Schuster <i>et al.</i> , 2014).
<i>Podocotyle lepomis</i> (Dobrovolny, 1939)	Opecoelidae	<i>Melanoides tuberculata</i>	Fish	Thailand (Krailas <i>et al.</i> , 2014).
<i>Procerovum cheni</i> (Hsu, 1950)	Heterophyidae	<i>Melanoides tuberculata</i>	Birds	China (Hsu, 1951).
<i>Procerovum varium</i> (Onji & Nishio, 1916)	Heterophyidae	<i>Melanoides tuberculata</i>	Birds	China (Hsu, 1951), India (Madhavi <i>et al.</i> , 1997; Umadevi & Madhavi, 2000; Arya <i>et al.</i> , 2016).
<i>Pygidiopsis genata</i> (Looss, 1907)	Heterophyidae	<i>Melanoides tuberculata</i>	Mammals	Egypt (Youssef <i>et al.</i> , 1987).
<i>Stellantchasmus falcatus</i> (Onji & Nishio, 1915)	Heterophyidae	<i>Melanoides tuberculata</i>	Birds, Mammals	Iran (Farahnak <i>et al.</i> , 2005).
<i>Transversotrema laruei</i> (Velasquez, 1958)	Transversotrematidae	<i>Melanoides tuberculata</i>	Fish	Thailand (Krailas <i>et al.</i> , 2014).
<i>Transversotrema patialense</i> (Soparkar, 1924)	Transversotrematidae	<i>Melanoides tuberculata</i>	Fish	India (Soparkar, 1924; Anantaraman, 1948; Rao & Ganapati, 1967; Nadakal <i>et al.</i> , 1969; Pandey, 1971; Madhavi <i>et al.</i> , 1997), Sri Lanka (Crusz, 1956), Israel (Ben-Ami <i>et al.</i> , 2005), Thailand (Ukong <i>et al.</i> , 2007).

Appendix D3: The *cox1* sequences used for the phylogenetic analysis during the present study. The GenBank accession numbers AY958764 and AY958760 were referred to as AF958764 and AF958760 in Genner *et al.* (2007); Percentage similarity is based on pairwise genetic distance calculations.

Snail species	Country	Site	Sample number	Similarity (%)	GenBank accession number	Reference
<i>Tarebia granifera</i>	South Africa	PHON	TP05	100	-	Present study
<i>Tarebia granifera</i>	South Africa	LIMP-2	M12	100	-	Present study
<i>Tarebia granifera</i>	South Africa	MOGA	M21a	100	-	Present study
<i>Tarebia granifera</i>	South Africa	LUVU	H39	100	-	Present study
<i>Tarebia granifera</i>	South Africa	OLIF-1	M61a	100	-	Present study
<i>Tarebia granifera</i>	South Africa	OLIF-2	M41	100	-	Present study
<i>Tarebia granifera</i>	South Africa	G-LETA	M71a	100	-	Present study
<i>Tarebia granifera</i>	South Africa	LETA	H59	100	-	Present study
<i>Tarebia granifera</i>	China	-	-	100	MZ662113	Yin <i>et al.</i> (2022)
<i>Tarebia granifera</i>	Thailand	-	-	100	MK000375	Veeravechsukij <i>et al.</i> (2018b)
<i>Tarebia granifera</i>	Thailand	-	-	98.2	MK000294	Veeravechsukij <i>et al.</i> (2018b)
<i>Tarebia granifera</i>	USA	-	-	98.2	MT671805	Tolley-Jordan and Triplett (2020)
<i>Tarebia granifera</i>	USA	-	-	98.1	AY958764	Genner <i>et al.</i> (2007)
<i>Tarebia granifera</i>	Singapore	-	-	96.9	AY958760	Genner <i>et al.</i> (2007)
<i>Tarebia granifera</i>	Singapore	-	-	96.4	AY958762	Genner <i>et al.</i> (2007)
<i>Tarebia lineata</i> (Gray, 1828)	India	-	-	83.9	KY511306	Jena and Srirama (2017)
<i>Tarebia lineata</i>	Malaysia	-	-	85.8	MW591171	Jamaluddin <i>et al.</i> (2022)
<i>Melanooides tuberculata</i>	Tanzania	-	-	85.6	AY791909	Von Gersdorff Sørensen <i>et al.</i> (2005)

Appendix D4: P-distance (pairwise genetic distance) given as the number of base pair differences between the sequences used for phylogenetic analysis during the present study.

	Sequence	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
A	M12_ <i>Tarebia granifera</i> LIMP-2	-	0	0	0	0	0	0	0	0	0	11	12	12	18	21	93	106	75
B	M21a_ <i>Tarebia granifera</i> MOGA	0	-	0	0	0	0	0	0	0	0	11	12	12	18	21	93	106	75
C	H39_ <i>Tarebia granifera</i> LUVU	0	0	-	0	0	0	0	0	0	0	11	12	12	18	21	93	106	75
D	M41_ <i>Tarebia granifera</i> OLIF-2	0	0	0	-	0	0	0	0	0	0	11	12	12	18	21	93	106	75
E	H59_ <i>Tarebia granifera</i> LETA	0	0	0	0	-	0	0	0	0	0	11	12	12	18	21	93	106	75
F	M61a_ <i>Tarebia granifera</i> OLIF-1	0	0	0	0	0	-	0	0	0	0	11	12	12	18	21	93	106	75
G	M71a_ <i>Tarebia granifera</i> G-LETA	0	0	0	0	0	0	-	0	0	0	11	12	12	18	21	93	106	75
H	TP05_ <i>Tarebia granifera</i> PHON	0	0	0	0	0	0	0	-	0	0	11	12	12	18	20	87	100	75
I	MZ662113_ <i>Tarebia granifera</i> China	0	0	0	0	0	0	0	0	-	0	11	12	12	18	21	93	106	75
J	MK000375_ <i>Tarebia granifera</i> Thailand	0	0	0	0	0	0	0	0	0	-	11	12	12	18	21	93	106	75
K	AY958764_ <i>Tarebia granifera</i> USA	11	11	11	11	11	11	11	11	11	11	-	0	12	19	22	77	87	79
L	MT671805_ <i>Tarebia granifera</i> USA	12	12	12	12	12	12	12	12	12	12	0	-	12	19	22	89	102	79
M	MK000294_ <i>Tarebia granifera</i> Thailand	12	12	12	12	12	12	12	12	12	12	12	12	-	17	20	87	100	71
N	AY958760_ <i>Tarebia granifera</i> Singapore	18	18	18	18	18	18	18	18	18	18	19	19	17	-	3	76	82	77
O	AY958762_ <i>Tarebia granifera</i> Singapore	21	21	21	21	21	21	21	20	21	21	22	22	20	3	-	79	85	77
P	MW591171_ <i>Tarebia lineata</i> Malaysia	93	93	93	93	93	93	93	87	93	93	77	89	87	76	79	-	13	76
Q	KY511306_ <i>Tarebia lineata</i> India	106	106	106	106	106	106	106	100	106	106	87	102	100	82	85	13	-	86
R	AY791909_ <i>Melanoides tuberculata</i> Tanzania OG	75	75	75	75	75	75	75	75	75	75	79	79	71	77	77	76	86	-

Appendix E



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ETHICS APPROVAL LETTER OF STUDY

Based on the review by the Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC), the Committee hereby clears your study as no ethical risk. This implies that the FNASREC grants permission that, provided the general conditions specified below are met, the study may be initiated, using the ethics number below.

Study title: The role of *Tarebia granifera* as an invasive in freshwater ecosystems of southern Africa.

Study Leader/Supervisor: Dr CW Malherbe

Student: JJ Pearson

Ethics number: N | W | U | - | 0 | 1 | 5 | 3 | 9 | - | 2 | 0 | - | A | 9
Institution Study Number Year Status

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application type: Single

Risk Category:

No Risk

Commencement date: 01/02/2020

Expiry date: 31/08/2023

General conditions:

The following general terms and conditions apply:

- The commencement date indicates the date when the study may be started.
- In the interest of ethical responsibility, the NWU-SCRE and FNASREC reserves the right to:
 - request access to any information or data at any time during the course or after completion of the study;
 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected;
 - it becomes apparent that any relevant information was withheld from the FNASREC or that information has been false or misrepresented;
 - submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or
 - new institutional rules, national legislation or international conventions deem it necessary.
- FNASREC can be contacted for further information or any report templates via Roelof.Burger@nwu.ac.za 018 299 4269

The FNASREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the FNASREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely,

Prof Roelof Burger

Chairperson Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)